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BIOLOGICAL BULLETIN

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BIOLOGICAL BULLETIN

HEREDITY FROM THE PHYSICO-CHEMICAL POINT OF VIEW.[1]

RALPH S. LILLIE.

Heredity, the power of reproducing its like, is a property of all forms of living matter from the lowest to the highest. Broadly speaking, whenever a universal property or mode of action is found, the presumption is that its basis is fundamentally *simple*, for repetition is characteristic of simple rather than of complex conditions and objects in nature: in general, the fewer the variables the more constant the phenomenon. We have therefore to seek for some general or fundamental structural or physico-chemical peculiarity of living things which enables their substance to build up substance of a similar kind. Any form of protoplasm acts as a center of construction of similar forms. At present we are not concerned with the further fact that this reduplication is not perfect, that varieties appear and that diversity has arisen in the course of evolution. This process of divergence is gradual, and even in mutants the differences from their parents are slight compared with the resemblances; the essential fact is that the type is preserved, and that normally the organism appears unable to construct living matter of other than its own kind. The main question is why any species of organism tends so strongly to retain its specific character.

Apparently the most universal property of living matter is its power of proliferation. Out of materials and energy taken from the surroundings it constructs more living matter of the same kind. In other words, the power of *growth* is innate; and if the available nutritive and other materials are sufficient the quantity of the specifically organized and active living substance tends continually to increase. The degree of increase possible under

[1] From the Laboratory of General Physiology, Clark University.

the conditions may be limited; and in fact most individual organisms and cells have definite limits of size, a balance being eventually reached where metabolic destruction balances construction; nevertheless the construction of new living substance, the essential process underlying growth, continues in all cases throughout life, and when it ceases, life ceases; the constructive process cannot suffer permanent interruption. Even where the organism as a whole seems to have reached its final dimensions there are always special cells or regions which when placed under appropriate conditions still show indefinite proliferative activity. In higher organisms such regions are represented more especially by the germinal epithelium; and a single detached cell from this region exhibits under certain conditions that unique property of definite and orderly proliferation known as development, which leads to the production of an organism similar in its minutest details to that of which the cell originally formed part. This is the chief form which the process of reproduction takes in higher animals, and such a manner of consideration shows that no fundamental distinction can be drawn between growth and reproduction. In many organisms almost any portion of sufficient size which is detached from the whole—whether by some physiologically normal mechanism like fission or as the result of operation—may continue its growth after isolation, redifferentiate, and eventually regain the form and physiological characters of the original stock. Whole plants may thus be reproduced from artificial cuttings, and the same is true of many lower animals (protozoa, hydroids, planarians). In such cases the distinction between growth and reproduction becomes ill-defined, and reproduction appears as essentially a form of discontinuous growth; and in an organism like yeast, where the growing cell-masses may either cohere in chains or fall apart into separate cells—apparently as the result of purely casual conditions—the distinction ceases to have more than a formal significance. If the cell-chain is regarded as the unit organism, increase in its length by budding is a matter of *growth*, if the single cell is so regarded, the same phenomenon becomes an instance of *reproduction*.

It is important for the purpose of the present discussion that the artificiality of this distinction should be recognized at the

beginning, in order that the nature of the essential problem should be clearly defined. Our aim is to analyze into its simplest physico-chemical terms—so far as this is possible at present—this power of specific construction, of structural and chemical synthesis, which is possessed by all forms of living matter. Reproduction and growth are different manifestations of the same proliferative process. Gametic reproduction is, to be sure, the most highly involved and specialized form of proliferation which we know; yet even here, as also in the case of a simple yeast-cell or bacterium growing in a culture-medium, the process of construction is accomplished through the action of the original germ in incorporating and transforming physically and chemically, in a definite and specific manner, certain materials (food, water, salts, oxygen) taken from the surroundings. The problem of just why the complex and highly organized living system thus built up from a particular species of egg-cell should exhibit its own specific structural, physiological, and chemical peculiarities, and why these should be identical with those of the parent, is one which can be solved in detail only by the special investigation of that particular species. But the fact that any such special development is a prolonged and complex process, involving a progressively increasing differentiation and at length reduplicating the parent form, does not alter its general character as proliferation. The fundamental *general* problem remains the same, whether the process under consideration is the formation of new yeast-cells or bacteria in a culture medium, or the development of a higher animal from a fertilized egg. In both cases material from the surroundings is transformed into living specifically organized substance of a constitution identical with that of the parent organism. And we have to ask whether it is possible, in the present state of our knowledge, to form any clear and consistent conception of the general nature of the physico-chemical conditions under which such a result is accomplished.

A simple concrete instance will define more clearly the nature of the problem to be solved. Consider the case of a single yeast cell introduced into a culture medium. The cell grows and forms buds; these give rise to other buds, and cell-chains are formed from which single cells detach themselves and pass

through similar transformations; eventually in place of the original cell there are thousands. The non-living material of the culture medium—consisting of water, sugar, ammonium tartrate, and inorganic salts of potassium, calcium, iron, and magnesium—has been transformed into complex and active living protoplasm of a specific chemical and structural organization. Each living cell exhibits the "germ-action" so characteristic of life—*i. e.*, acts as a center of chemical and physical transformation of a definite kind by which more and more yeast protoplasm is formed. We may note here the analogy—to which we shall return—with the process of specific accretion by which a crystal introduced into a super-saturated solution increases its size and becomes a center of deposition of more crystals of the same kind. But the analogy is incomplete; the living organism does not merely change the physical state of the substances which it takes in from the surrounding solution; it also modifies them chemically in the most profound manner, and from the above simple materials it builds up proteins, lipoids, fats, glycogen, and a multiplicity of other substances not present in the culture medium. Further, not only are these substances synthesized, but they are distributed throughout the growing mass of protoplasm in a perfectly definite manner, partly as solid structural material, partly as dissolved or other material serving for energy-production or some form of metabolism.[1] Each one of the specific organized structures thus produced, the yeast cells, reduplicates the physiological activities as well as the structural characters of the parent cell. The result is that the non-living material of the surroundings is transformed or worked over into organized living material of a predetermined type, *i. e.*, the transformation is *specific* and depends upon the nature of the germ originally introduced into the medium. Both growth and heredity are exemplified in their simplest manifestations; and we see again that these terms do not signify two objectively different processes, but merely two aspects of the same process—"growth," the quantitative term, denoting the increase in the total mass of living substance, while "heredity" emphasizes the specificity of the process and its dependence upon the parental character.

[1] *I. e.*, the problem of *differentiation* is inseparable from the problem of growth.

It is possible to say that each yeast cell *inherits* its properties from the mother cell; but such a phrase serves merely to indicate that the character of the transformation depends upon the character of the germ or proliferating organism itself, rather than upon the character of the material which is transformed under its influence. A mould or bacterium in the same medium effects a totally different kind of transformation, but one equally specific and equally true to the character of the introduced germ.

In general the possession of this automatic property of specific structural and chemical synthesis constitutes the most fundamental distinction between the living and the non-living material systems found in nature. We observe that all organisms and all living cells without exception possess this power; they transform certain inert materials selected from the surroundings into their own characteristically organized and physiologically active living substance. The materials thus incorporated and transformed differ widely in their character and accessibility in different organisms; at the one extreme are green plants living on carbon dioxide, salts, and water; at the other extreme is man in his complex social environment. But this difference of degree does not alter the fundamental identity of kind. Wherever we find life we find exhibited this unique property of specific construction or synthesis, whether the product of the construction is simple or complex.

This property also manifests itself in another and less evident manner. The specific organization of any animal or plant, with the associated physiological activity, not only *originates* in this manner as the product of growth from the parent organism, but once reached, it has to be *maintained*. And this maintenance involves the activity of specific construction in just as full a sense as does growth or development from the germ. During life the organized living substance is continually being destroyed, and must as continually be replaced. This becomes especially evident in certain of the lower animals whenever the food-supply is withdrawn; the quantity of living substance then undergoes progressive reduction, which may proceed until only a small fraction of its original quantity is left; a planarian may thus be reduced by starvation to a quarter of its original length without

injury, but disorganization and death follow further reduction unless food is restored. A similar loss of living substance through the destructive metabolism inseparable from the life-process takes place in all cells, although its rate and its possible limits vary in the different cases—apparently because of the unequal resistance of different structural elements to regression of this kind; thus in higher animals the loss during starvation is great in voluntary muscle and small in the heart and nervous system. In all cases the specific structural material appears subject to a certain continual breakdown of this kind; even when the non-nitrogenous food-supply is ample for energy-requirements it is found impossible to reduce the nitrogen metabolism—the index of destruction of protein, the specific structure-forming substance—below a certain well-defined minimum. This can only mean that the structural or organized material is subject to continual destruction, and that maintenance involves its continual replacement. This process of replacement is specific, in precisely the same sense in which the growth-process is specific. We must therefore recognize that maintenance involves the activity of specific chemical and structural synthesis in the same sense as does growth. When the construction of organized substance balances destruction there is equilibrium—the condition corresponding to nitrogenous equilibrium in higher animals; any excess of construction leads to growth, of destruction to regression. We see once more that what is essential to continued life is the specific synthetic activity of the protoplasm; when this ceases, life ceases. Claude Bernard expresses this necessary dependence of life upon synthetic or creative processes in the phrase, "life is creation." It is clear that the process of specific creative synthesis which lies at the basis of heredity is inherent in the life-process in all of its forms. The problem of heredity is not a problem to be dealt with by itself; it becomes identical with the most fundamental problem of general physiology, the problem of how living protoplasm is synthesized from non-living matter.[1]

[1] All of this is clearly recognized by Claude Bernard; *cf.* "Leçons sur les phénomènes de la vie," Vol. 2, p. 517, where he summarizes his general view as follows: "The synthetic action by which the organism thus maintains itself [*i. e.*, by a combination of chemical and formative synthesis] is at bottom of the same nature as that by which it repairs itself after it has undergone mutilation, or still further,

The general physiological problem presented by the phenomena of growth and heredity thus reduces itself to these terms: what are the essential physico-chemical conditions upon which this power of specific construction depends? It seems clear that only a thorough knowledge of the conditions determining the special type of metabolism involved in the process, and especially of its constructive side, can answer such a question. The living organism or cell is primarily a metabolizing system. Growth is the expression or result of a process of metabolism; both the material and the energy required for growth come from outside; within the organism they are transformed in such a manner as to build up an organized system of predetermined kind, the seat of chemical and physical processes which maintain the system and enable it under appropriate conditions to increase in size or to produce other similar systems leading independent life.

In all organisms constructive metabolism involves the synthesis of a multiplicity of new chemical compounds from the food and other materials furnished by the surroundings. The food-materials are typically non-specific in their chemical nature, *i. e.*, they show no relation to the specific character of the organism utilizing them; they are either chemically simple in themselves, or become so during incorporation. In animals, where the organism receives part of its food in highly specific form, as protein, all specificity is invariably lost in the hydrolytic processes of digestion; the material before becoming available for nutrition is reduced to a form in which it can be utilized indifferently by all cells. This non-specificity of the food-materials, as they reach the cells, is in striking contrast with the specificity of the compounds built up from them within the cells. Reduction to a simple or non-specific state is thus the indispensable preliminary to the constructive process. It is therefore highly significant that the chief structural colloids, the proteins, are so readily transformed from the specific to the non-specific state.

by which it grows and reproduces itself. Organic synthesis, generation, regeneration, maintenance, healing of wounds, are different aspects of an identical phenomenon, are varied manifestations of the same agent. . . ." In the Presidential address of J. S. Haldane before the British Association in 1908 (Nature, Vol. 78, p. 555) a similar point of view is expressed, *e. g.* "nutrition itself is only a constant process of reproduction .... Heredity is for biology an axiom and not a problem."

The universal presence of proteases in cells seems to be the expression of this necessary condition, since in all cells the structural proteins are capable (under certain conditions) of regression and translocation, *i. e.*, of being utilized as food-material elsewhere—the proteins of one region of the cell or organism acting as reserve, so to speak, for construction at other regions. Such a condition is apparently necessary for the normal regulation of cell-structure and activities. For the continuance of normal cell-activity a proper ratio between structural and metabolizing substance must be preserved;[1] hence in every cell the conditions must be present for reducing proteins and other materials to a non-specific and diffusible form, in addition to the conditions for specific synthesis.

By the synthetic activity of the protoplasm these relatively simple substances are united and chemically remodeled so as to form a variety of more complex compounds of which the most individualized and specific are the proteins. The term *specific* is here used as meaning peculiar to the particular organism or cell under consideration, and not occurring elsewhere. It is not a coincidence that living organisms, the most complex systems, in the structural sense, occurring in nature, are also the most complex in the purely chemical sense; and all of the evidence indicates that the structural complexity is the expression or consequence of the chemical complexity.[2] The essential reason for this appears to be that a high degree of chemical specificity or individualization is the necessary prerequisite for structural complexity, and that chemical specificity depends largely upon peculiarities of stereochemical configuration. The number of individualized isomers in the case of any organic compound increases rapidly with increase in the number of asymmetric carbon atoms in the molecule. Hence the proteins, formed of linked amino-acids, most of which are asymmetric compounds, exhibit the possibilities of chemical individualization to a greater degree than any other known class of compounds. It is further significant that proteins which are specifically distinct chemi-

[1] *E. g.*, in a starving protozoön or planarian the normal structure and proportions are preserved, in spite of the decrease in size.

[2] Similarly with structural diversity, whether in the same organism or in different organisms. A corresponding chemical diversity is implied.

cally, although otherwise closely similar—*e. g.*, hæmoglobins from different species—tend to form crystals, *i. e.*, structural aggregates, which are specifically distinct in their form-characters.[1] A definite relation between the chemical specificity and the structure-forming properties of these colloids is thus indicated. The relation of proteins to organic structure therefore requires special consideration in any theory of heredity.

The proteins form the colloids out of which, together with certain other associated materials, chiefly lipoid, the more permanent or stable, *i. e.*, "structural" portions of most living organisms, and especially the cell-structures, are built up. The relations of these substances to the specific characters of the organism must for this reason be recognized as peculiarly intimate, even though we are not yet in a position to understand the exact relationship between a particular type of structure in an organism and the specific peculiarities of the protein composing that structure. The available evidence indicates, however, that a definite relationship of this kind does exist. We know for example that the presence of foreign proteins is often incompatible with the preservation of normal structure in cells; the cytolytic action of foreign blood-sera, and the formation of specific cytolysins when cell-proteins are used as antigens, show that so fundamental a character as the semi-permeability of the plasma-membrane in a cell is dependent upon the specific peculiarities of its constituent proteins. And in all cell-structures it is probable that a similar relation exists. Such facts indicate clearly that the specific structure of a cell or organism depends upon the chemical specificity of its structural proteins. Now the term specificity, as applied to the individualized character of an organic species, has its morphological and physiological as well as its chemical significance: *i. e.*, each species has its own special form and structure and its own characteristic modes of activity and behavior, as well as its own distinctive and unique chemical composition. But according to the present conception it is the *chemical* specificity which forms the basis of the other two, and

[1] *Cf.* Reichert and Brown: "Differentiation and Specificity of Corresponding Proteins and other Vital Substances in Relation to Biological Classification and Organic Evolution: the Crystallography of Hæmoglobins." Carnegie Institution of Washington, 1909.

chemical specificity is primarily the property of the proteins. Other biochemical compounds appear to be chemically the same wherever found, but the proteins vary in their specific character from species to species. Moreover, physiologically corresponding or "homologous" proteins are more nearly alike in their chemical and physical characters the more closely related the species are from which they are taken. There is thus a general parallelism between the degree of chemical relationship exhibited by homologous proteins from different organisms, and the degree of biological relationship existing between the species. The indications of this are too various to present in detail in this brief paper, and the evidence has recently been reviewed in an admirable manner by Loeb in his "Organism as a Whole."[1] The specificity which such proteins exhibit when used as antigens, *e. g.*, in the formation of precipitins or specific cytolysins, or in the phenomena of anaphylaxis, shows clearly that the proteins of one species are chemically distinct from the corresponding proteins of even nearly related species, and still more distinct from those of more distant species. Nuttall's well-known work shows that the ability of a given precipitin to react with and precipitate its corresponding protein from another species is a close indication of the degree of blood-relationship between the species under consideration.[2]

Apart from these facts, whose significance in relation to the present problem is now well recognized, there are other evidences of chemical specificity in proteins that offer clearer indications of the nature of the connection between the chemical character of a protein and the character of the structures which it forms in the living cell. The work of Reichert and Brown has shown that hæmoglobin crystals from a given species exhibit form-characters which are definite and specific for the species. This means that when the protein separates from solution in the process of crystallization the molecules, as they unite to form larger crystalline aggregates, by degrees build up structures with definite form-characters—the typical forms of one species exhibiting constant differences from those of other species, even of

[1] *Cf.* Chapter 3: "The Chemical Basis of Genus and Species."

[2] Nuttall, "Blood Immunity and Blood Relationships." Cambridge University Press, 1904.

the same genus. The growing crystal takes on a definite "species-specific" form, in a manner suggesting a close analogy to the growing germ; this definite form is the expression of the specific properties of the protein molecule, and is presumably dependent upon its special stereochemical configuration. It is well known that molecules of similar stereo-structure tend to segregate in the process of crystallization; thus in the crystallization of a racemic tartrate from its solution one group of crystals is formed exclusively—or at least predominantly—of molecules of the dextro-tartrate, the other of the lævo-tartrate, although in respect to solubility and other physico-chemical properties the two compounds are identical. In such an instance it is quite certain that similarity of stereo-structure is the critical factor determining the union of the dextro-molecules to form a definite crystalline aggregate specifically distinct from that formed by the lævo-molecules.[1]

The physiological properties of the two stereo-isomers are correspondingly unlike; fermentability and related properties (such as general assimilability and pharmacological action) have been shown to differ markedly in a large number of pairs of asymmetric compounds, a clear proof that the activity of living protoplasm is largely conditional upon the specific space-relations of the atoms composing the physiologically active molecules. This is particularly true of compounds entering into metabolism: thus we know that enzyme action is determined by stereo-structure. Perhaps the clearest proof that specific constructive metabolism is similarly determined is furnished by the specific character of the metabolic response following the introduction of protein antigens into the organism—*i. e.*, by the specific character of the anti-bodies produced. These new compounds, evidently synthesized by the living cells, exhibit specific chemical

[1] Thus the introduction of a crystal of the lævo form into a supersaturated solution of racemic tartrate causes the separation of lævo-tartrate alone; similarly dextro-crystals separate out dextro-tartrate (*cf.* Gernez: Comptes rendus, 1866, Vol. 63, p. 843). The recent work of Marc indicates that in general crystallization is preceded by an adsorption; and that crystals, when used as adsorbents, adsorb by preference substances which crystallize in a similar form. *I. e.*, the similarity in the spatial configuration of the molecules is what determines their union to form larger aggregates (*cf.* Marc: Zeitschr. physik. Chem., 1913, Vol. 81, p. 641; also *ibid.*, 1911, Vol. 75, p. 710, and earlier papers there cited).

properties which are directly determined by the specific properties of the foreign protein introduced. The intimate relationship between the phenomena of immunity and the phenomena of normal assimilation has been pointed out by Ehrlich and others. And since heredity is primarily a matter of assimilation—*i. e.*, construction of the like—it seems clear that the construction of specific protein in the normal processes of growth is the expression of a similar determination of specific constructive processes in the cell by means of the proteins *normally* present. In other words, *the structural proteins already present must determine the production of similar proteins.*

The above tendency of structurally similar compounds to form aggregates when they separate from solution is probably the essential reason why the proteins native to the cell—already forming part of its structure—undergo increase in quantity, with the result that the cell grows. Nothing less than this is to be inferred from the fact that proteins form the basis of specific structures, and that the preservation of the normal characters of any living cell depends upon the continual formation and reformation of these particular compounds. Those proteins which are already laid down as structure within the living cell are thus to be regarded as acting as centres of deposition of further protein identical in composition and configuration. It seems necessary also to conclude that these same structural proteins directly control or guide the actual synthetic process by which more protein of the same kind is built up, presumably by the dehydrolytic condensation of the amino-acids present in the protoplasm. The precise and specific mode of union corresponding to any particular structural protein would then be determined by the specific character of the protein itself. Such a conception would correspond to that of specific catalyzers—the usual manner of conceiving these phenomena at present—the only difference being that the structural protein would itself play the part of catalyzer. Such a process would constitute a form of autocatalysis; the resemblance of growth to an autocatalytic process has in fact been emphasized by Loeb, Robertson, and others.[1] The structural

[1] *Cf.* J. Loeb, *Biochem. Zeitschr.*, 1906, Vol. 2, p. 41. T. B. Robertson, *Arch. f. Entwicklungsmech.*, 1908, Vol. 25, p. 581. Wfg. Ostwald: Roux's *Vorträge und*

peculiarities of such deposits, and hence the specific morphological characteristics of the cell, would be determined by the specific chemical characteristics of the constituent proteins, in a manner analogous to that by which the special form of a hæmoglobin crystal-aggregate is determined. And the special character of the structure thus laid down would determine the special character of the metabolism, and hence the special type of physiological activity exhibited by the cell. This last conclusion seems inevitable, since the source of this activity is metabolism, which in all living systems is under the control of structure. In other words, the formation of a specific structure in the protoplasmic substratum will necessitate a correspondingly specific type of metabolism, since the nature and rate of the metabolic chemical reactions are controlled by the structural conditions present; the dependent physiological or functional manifestations must therefore also be specific.

We are thus led to conceive certain features of the organic formative process in a somewhat definite manner, which may be summarized briefly as follows: The specific characters of any animal or plant are determined ultimately by the specific characters of its structure-forming proteins. The developing germ or the growing organism synthesizes specific proteins, and these, since they determine the structural and hence the physiological peculiarities of the organism, form the basis of its special character as an organic species. Accordingly one of our most fundamental problems is to determine why the cell builds up proteins of its own specific type. The essential problems of heredity and reproduction center here. As we have seen, heredity is exemplified whenever one yeast-cell or bacterium gives rise to another; also whenever any cell grows and increases its living organized material. This increase in living material is indispensable for the continuance of the species, and for this reason we may characterize growth as the fundamental life-process, and the problem of growth in its most general aspect as identical with the problem of heredity. The factors of growth are the factors of heredity.

Aufsätze, Heft 5, 1908. Chodat made a similar suggestion for plant growth in 1905 (*cf.* D'Arcy Thompson: "Growth and Form," Cambridge Univ. Press, 1917, p. 132).

Such a conclusion directs our particular attention to the general nature of the conditions controlling growth.

Growth and development may be controlled to a greater or less degree by various artificial means; and much of experimental embryology is concerned with modifying the rate and character of either process. In this way many definite hereditary characters may be profoundly altered, or in some instances their appearance may be altogether suppressed. A simple and instructive instance is described by Loeb.[1] The sea-urchin egg will develop to the gastrula stage in a balanced solution of sodium, potassium, and calcium chloride; if in addition to these salts some sodium carbonate is present, the skeletal spicules may form and a pluteus larva develop, but not otherwise. The skeleton is an inherited character; its formation, however, is dependent upon the presence of sodium carbonate in the surrounding medium, as well as upon the organization of the germ; the necessary carbonate must be furnished to the germ from without, or the specific formative process is unable to take place. Such a result is not difficult to understand. Development, like growth, is a matter of metabolism, and primarily of constructive metabolism; hence it is influenced by any condition that influences metabolism; accordingly the presence or absence of food, oxygen, water, salts, vitamines, hormones, as well as the conditions of temperature, may each and all have determinative relations to the total process. It is significant, however, that the specific characters of the organism, those which, according to the present view, express the chemical specificity of its structural proteins, seem never to be essentially altered by such changes of condition, although their appearance may be prevented or the degree of their development modified. Whatever structural characters appear in development are those characteristic of the species; this statement may be qualified to the degree required to take into consideration the facts of mutation (these suggest that under exceptional conditions new structural proteins may be synthesized); but the essential fact which we wish to express is the tenacity with which the organism preserves its specificity. At least this specificity can be modified, if at all, only by gradual

[1] J. Loeb, *Amer. Journ. Physiol.*, 1900. Vol. 3, p. 441.

degrees; and any theory of growth or heredity must assign some definite basis for this characteristic conservatism. We have suggested above that this basis is the tendency of similarly constituted compounds to segregate in the formation of aggregates, and in this way to form structures which determine the direction of metabolism. But such an hypothesis explains only the *resemblance* of an organism to its parent; it does not indicate how the proliferative process itself is carried out. It appears, in fact, that two separate groups of problems are involved in the theory of heredity—the one relating to the conditions determining the resemblance to the parent stock, the other relating to the nature of the physiological mechanism by which the living substance (apart from its special nature) increases its quantity or *grows* at the expense of materials taken from the surroundings. Such growth is a physiological activity requiring the expenditure of energy, and it cannot be considered apart from its relation to the other physiological, *i. e.*, functional, activities of the organism. The nature of this latter relation now calls for special consideration.

The general significance of the normal functional activity of the living system as one of the chief factors in the formation of its characteristic structure, or in structure-forming metabolism generally, has been insufficiently regarded by writers on heredity. In general, any normally active tissue maintains itself or grows, while an inactive tissue remains stationary or undergoes regression, even if supplied with an abundance of oxygen and food-material; this last is well shown in a voluntary muscle whose innervation has been interrupted. It is clear that increased functional activity involves an increase of constructive as well as of destructive metabolism; and conversely the subnormal metabolism accompanying inactivity is associated with subnormal construction, which may even fall below destruction, with regression or atrophy as a result. The control which function exercises upon the building-up of living structure is seen perhaps most clearly in higher organisms, and especially in tissues like voluntary muscle, whose activity is intermittent and subject to much variation. In this case the effects of exercise in promoting growth and of disuse in causing regression are

familiar to all; and essentially similar conditions are found in other tissues and organs. Functional hypertrophy following excessive activity, and regression or atrophy following prolonged inactivity, are both well-known phenomena; for example, compensatory hypertrophy in heart muscle is a frequent result of valvular lesions, and one kidney increases in size if the other is removed. In general it would appear that any physiological function can reach and preserve its highest perfection only through continual repetition; and this very condition implies that decline must follow inactivity if the latter is prolonged beyond a norm. And since every function has some organized structure as its correlate, the same considerations apply to whatever structures are concerned in the function in question. The modifications which the central nervous system undergoes in association with the process of learning afford instances of an essentially similar kind; practise facilitates the repetition of any complex voluntary action, *i. e.*, perfects the structural and other adjustments underlying the function;[1] while any accomplishment, intellectual or other, declines with disuse. These examples may suffice to illustrate the general principle under consideration. It seems clear that the physico-chemical mechanisms—whatever their nature may be—controlling functional activity are in some intimate relation to those determining growth. The above facts seem to imply that both classes of physiological processes are simultaneously and equally under the control of some more general set of conditions characteristic of living substance in general. We shall now consider this possibility in more detail.

Claude Bernard has pointed out how essential it is in any living system—if the system is to continue to exist—that there should be a relation of interdependence between the processes of destruction and of repair, of such a kind that any destructive or dissimilatory process sets in motion automatically the contrary process of repair.[2] All functional activity involves break-

[1] This is the basis of the phenomenon of *memory*. Hering has discussed briefly the relations between memory and heredity in his well-known address on "Memory as a General Function of Organized Matter," Vienna Academy, 1870; English translation by Open Court Publishing Company, Chicago, 1897.

[2] "Leçons sur les phénomènes de la vie," Vol. 1, Chapter 3.

down of organized material, and apparently some disintegration of cell-structure then always takes place, for protein metabolism is increased—even though slightly under good nutritive conditions—by increased muscular work.[1] Hence for normal regulation of cell-activity it is essential that a compensatory or constructive series of processes should be aroused into action by the same conditions that stimulate or call forth the destructive or energy-yielding activity. "Functional breakdown in living material is itself the precursor and instigator of the renovation accomplished by the formative process, which works silently and obscurely in the interior of the tissue" (*i. e.*, without evident external manifestation, in contrast to the destructive process). "Losses are thus repaired as rapidly as they are caused, and since equilibrium tends to re-establish itself as soon as it is destroyed, the normal composition of the living body is maintained."[2] Bernard also recognizes that this process of restitution

[1] It is now amply demonstrated that increased muscular work in higher animals leads to little or no increase in breakdown of protein (as indicated by N-excretion), provided the non-nitrogenous food-constituents are sufficient in quantity, especially the carbohydrates. If the food contains sufficient protein for maintenance, but carbohydrate and fat are deficient, there may be a considerable increase in N-excretion, but typically not enough to account for the increased energy-production on the basis of oxidation of protein; in this case the surplus of energy comes from the non-nitrogenous reserves of the organism. Carbohydrate is especially effective as a protein-sparer, a fact indicating that in the construction of protein it plays an essential part. It is also the chief source of muscular energy; and the fact that vigorous muscular work, involving active consumption of sugar, is the chief condition for the normal growth of the tissue, shows that the energy required for this growth—*i. e.* for the chemical and structural syntheses involved—is derived from the oxidation of sugar. The possible metabolic changes concerned in this process cannot be considered in an article like the present. But that carbohydrate is essential for the assimilation of amino-acids and other nitrogen compounds in both animals and plants is indicated by a large body of recent and older investigation. Thus for the assimilation of amino-acids by yeast and moulds sugar is indispensible (*cf.* the series of papers by F. Ehrlich, *Biochem. Zeitschr.*, 1906, Vol. 1, p. 8; 1908, Vol. 8, p. 438; 1909, Vol. 18, p. 391; 1911, Vol. 36, p. 477); similarly in higher plants the synthesis of protein from amides in germination requires the presence of carbohydrates (for a brief summary of the facts *cf.* Jost's "Physiology of Plants," p. 175). The work of Loewi, Lüthje and others has shown the great importance of carbohydrates in the synthesis of protein from amino-acids in higher animals; there is also clear evidence that derangement of carbohydrate metabolism (*e. g.*, in pancreatic or other diabetes) interferes very directly with the synthesis of protein (*cf.* Chapter 9 of Cathcart's "Physiology of Protein Metabolism" for a general review of this subject and literature).

[2] "Leçons sur les phénomènes de la vie," Vol. 1, p. 127.

involves not only a chemical synthesis, but also a morphological synthesis, *i. e.*, a rebuilding of organized structure. The constructive and the destructive processes are inseparable; synthesis is life, whether during rest or activity. Hence the rate of construction must be regarded as ultimately under the same kind of control as the rate of destruction, even though the latter is more obviously subject to modification under the usual conditions of life (as in stimulation, voluntary action, etc.).[1] As instances of the initiation of organic construction by conditions whose primary effect is to stimulate, *i. e.*, to *increase* the energy-yielding dissimilation, Bernard cites the awakening of dormant germs or hibernating animals by rise of temperature or other stimulating condition;[2] the resumption of growth and other processes involving increased assimilation illustrates the constant, though it may be indirect, nature of the interconnection. An apposite present day-illustration of the same phenomenon would be the initiation of development in unfertilized eggs by a temporary cytolytic action. But one does not need to search for instances; the reciprocal interdependence of assimilation and dissimilation is seen everywhere in organisms; how essential this relation is for the preservation of life appears, for example, in the general fact that in all animals increased muscular or other activity hastens the onset of *hunger*, *i. e.*, of the condition necessary for supplying the raw material for construction. The maintenance of a balance between the two kinds of metabolic processes constitutes probably the most fundamental of the various types of organic regulation.

There is no doubt that a general regulatory condition of this kind exists in all organisms; the problem is to determine the essential physico-chemical conditions upon which it depends. We must regard the living system primarily as one in which the synthesis of both chemical substance and organized structure is controlled by functional activity. And during the growth and development of the system, *i. e.*, at the periods when synthetic

[1] Bernard gives direct experimental evidence of the identity of the conditions controlling growth with those controlling stimulation by showing that growth (*e. g.* in seedlings) may be anæsthetized under the same conditions as the different forms of irritability. *Cf.* his chapter on Irritability, Leçons, Vol. 1, p. 267.

[2] *Cf.* Leçons, Vol. 1, Chapter 2, p. 110.

activity predominates, a similar dependence of formative activity on function must exist. This is the kind of relation emphasized recently by Child, when he describes the developing organism not as being first constructed and then functioning, after the manner of a machine, but as constructing itself *by* functioning.[1] Growth and development are peculiar in that specific construction overbalances destruction, and that the synthesized material, as it accumulates, adopts a definite organization. But this accumulation of structure is itself the expression or result of active functioning, in which energy is freed, just as growth is such an expression in a muscle which has been exercised. Apparently we must conclude that part of this energy is expended in the work of synthesizing and arranging the specific structural material of the organism or cell. We have already seen that this specific material consists essentially of protein. We are thus brought back to the question: what are the conditions under which protein is synthesized in the living cell and deposited as structure?

No very definite or certain answer can be given at present to this question. But we seem to be in a position to rule out certain possibilities, and perhaps to affirm others. First we must note more particularly the significance of the long recognized fact that many vital syntheses require the addition of energy to the synthesized compounds; this is seen, for instance, in the formation of fats from proteins and carbohydrates. Now since such syntheses, where compounds of higher chemical potential are built up from those of lower, take place continually in all cells, it appears highly probable that their conditions are also the main conditions of synthesis in general, and that a subordinate importance is to be attached to the purely enzymatic type of synthesis. Both experience and theory show that the latter is limited to the formation of compounds in which little change of energy accompanies the transformation;[2] hence it is plainly insufficient to meet the normal requirements of constructive metabolism. Some kind of mechanism would seem to be indicated in which energy derived from oxidation or other chemical source is applied

[1] C. M. Child, "Individuality in Organisms." University of Chicago Press, 1915, p. 16.

[2] *Cf.* Höber's "Physikalische Chemie der Zelle und der Gewebe," 1914, p. 677.

to perform the work of chemical and structural synthesis. Enzymes may facilitate or direct certain kinds of combinations, and in this way they may be important as accessory factors in the constructive process; but the essential controlling factors are evidently of a more active, *i. e.*, work-performing, kind. And since these factors vary in their activity with function, it follows that the conditions controlling the degree and rate of functional activity—*i. e.*, generally speaking, the conditions of stimulation —must at the same time be the conditions controlling the specific constructive processes.[1] It is true that construction does not always run parallel with destruction, the rate of which may often temporarily exceed that of repair; and at times nutritive or other conditions may render complete replacement impossible; or at other times construction may preponderate, as in growth. Nevertheless an interdependence of the kind indicated unquestionably does exist; and apparently we must infer that part of the energy freed in the oxidation (or other energy-yielding decomposition) which performs the work of function is applied, in some manner as yet unknown, to build up the material required for maintenance or further growth.

If we adopt this general hypothesis, we must reject as entirely insufficient the conception of growth as being analogous to a process of crystallization, or as being determined by syntheses of the enzymatic kind; and we are led to look for some other type of process in which the formation and deposition of structural material is controlled by energy set free in chemical change. This process must be capable of variation in rate, of interruption and renewal, and of reversal,—if it is to correspond to such

[1] This is indicated by Bernard's already cited observation that anæsthetics arrest growth-processes reversibly in the same manner as they inhibit stimulation or other forms of functional activity,—a fact suggesting that physico-chemical changes of the same nature control both growth and the response to stimulation. If this is true, it seems probable that the structures primarily concerned in stimulation are also those primarily concerned in construction, *i. e.*, the site or *locus* of both constructive and destructive processes is the same, the two representing reverse phases of the same process. On such a view the idea that special regions of the cell (*e. g.*, the nucleus) are the exclusive seat of syntheses would have to be abandoned. There is, however, much evidence that the nucleus is necessary for the continuance of synthetic processes; possibly it gives rise to certain substances which are required for the maintenance of the structures more immediately concerned in the specific syntheses.

features of organic growth as the variation of the latter with the conditions (*e. g.*, temperature or the supply of food and oxygen), its dependence on function, and the possibility of regression. Further, it must be a process not necessarily peculiar to living organisms, although apparently taking place under especially favorable conditions in these systems, and it must be able to effect either chemical decompositions or syntheses. All of these peculiarities seem to point to some physico-chemical process of the general nature of electrolysis as underlying the synthetic activity of organisms. In other words the possibility presents itself that electrosynthesis is the chief method of construction in the living system.

The main physiological facts which appear to me to favor this hypothesis are briefly as follows. All functional activity is associated with the formation of electrical circuits between different regions of the cell or organism. The currents of these bioelectric circuits are in many cases sufficiently intense to produce marked physiological effects upon other cells or tissues (stimulation, etc.), and presumably they have similar effects upon other regions of the same cell; the transmission of the effects of local excitation appears in fact to be due to an action of this kind. In general we observe vital functions to be profoundly influenced—accelerated, inhibited, or initiated—by electrical influence; and since function involves specific construction, the constructive process must be subject to similar influence. Experimental data upon the influence of the electric current on growth processes are as yet comparatively few; but galvanotropism is well known in plants, and with properly devised experimentation could probably be shown to be widespread. The control of growth processes by the electric current offers in fact a largely untouched field of investigation, which would probably yield results not only of great theoretical interest but of practical importance as well (*e. g.*, as possibly affording a means of controlling malignant growths, etc.).

But perhaps the clearest indications that the organic formative processes are under the control of electrical conditions are seen in the striking resemblances which certain types of electrolytic deposit show, both in their structural character and in the con-

ditions influencing their formation, to the structures arising by normal growth in organisms. The two types of phenomena exhibit many close and, as it seems to me, highly significant parallels. Inorganic structures resembling vegetative growths are seen not only in the formation of lead or tin "trees" from metallic zinc immersed in solutions of salts of these metals, but they are shown in an especially striking form in those cases where the local process of electrolysis gives rise to precipitates which form coherent membranes or otherwise exhibit colloidal character. In a recent paper I have described the structure and conditions of formation of such precipitation-structures in some detail, and have discussed the reasons for their resemblance to organic growths.[1] In solutions of potassium ferricyanide, especially those containing egg-albumin or other protective colloid, pieces of metallic iron, zinc, or copper produce characteristic filamentous or quasi-cellular outgrowths, consisting of precipitation-membranes of the ferricyanide of the metal used, which resemble strongly certain definite organic types of growth like mould-hyphæ. These structures grow out into the solution from the anodic regions of the metallic surface; hence their formation may be accelerated, retarded, or suppressed at will by varying the character of the local circuits determining the rate at which the ions of the metal enter solution. A region of (*e. g.*), iron which is actively forming precipitation-filaments will at once cease this action if it is rendered the cathode in another intercepting local circuit,—*e. g.*, by the contact of zinc or other baser metal at a neighboring point; or conversely it may be rendered still more active—*i. e.*, more strongly anodic—by increasing the intensity of the local circuit in which it acts as anode,—*e. g.*, by the adjacent contact of a nobler metal (*e. g.*, platinum) or carbon.[2] It is especially to be noted that the relations between the different electrode-regions of such local circuits are *reciprocal*, as regards the character of the chemical changes there taking place; this is inevitable, since in general the electrochemical processes at any anode are the reverse of those at the cathode. Hence the formation of filaments at one region of the metallic surface appears to have the effect of *inhibiting* their formation at another ad-

[1] BIOLOGICAL BULLETIN, 1917, Vol. 33, p. 135.

[2] *Loc. cit.*, p. 148.

joining region. A similar reciprocality of influence is especially characteristic of physiological processes, such as excitation and inhibition, *e. g.*, in the central nervous system; and it is also well known to be characteristic of various processes of growth and regeneration in both animals and plants.[1] This is why (for example) cutting off a tubularian head enables an adjoining region of the stem to form a new hydranth; the region where the new growth takes place has been removed (by the operation) from the inhibiting influence of the original hydranth. Similarly a short piece of iron wire which is in contact with a piece of zinc will not form filaments in ferricyanide solution until the zinc is detached or otherwise rendered inactive. Cutting away the zinc thus initiates the development of filaments from the iron;[2] the structure-forming process had previously been inhibited by the activity at the zinc, which on account of its greater tendency to send ions into solution alone forms filaments while the two metals are in contact. To express the matter biologically: the zinc seems actively to appropriate the available structure-forming material (ferricyanide), and in so doing prevents the iron from utilizing this material to form filaments. Similarly the hydranth, with its higher rate of metabolism, acts as the chief structure-forming region in the tubularian, and inhibits structure-formation of the same kind in its vicinity.[3] It corresponds, in this sense, to the anodal metal in the local electrical couple of zinc and iron. The growth-initiating consequences following physiological isolation—to use Child's concise and illuminating expression[4]—may thus be instructively simulated by means of an inorganic model of this kind.

These and similar parallels appear to indicate that *the same type of process* is concerned in the structure-formation in the two kinds of system, otherwise so entirely unlike in character.[5] If

[1] *Loc. cit.*, pp. 156, 163.

[2] *Loc. cit.*, pp. 152 *seq.*

[3] This is an example of the dominance or control of formative processes by those regions having the highest rate of metabolism: *cf.* Child's "Senescence and Rejuvenescence," Chapter 9, p. 210; also "Individuality in Organisms," Chapter 5.

[4] *Loc. cit.*

[5] It is a question whether an electric current passing between any semi-permeable water-insoluble phase and the adjoining aqueous solution can do so otherwise than by a process of ionization (or deionization) at the boundary, *i. e.*, by a process

this is true, the transmission of the structure-forming or growth-inhibiting influence is in both cases due to the formation of electrical circuits between the influenced and the influencing regions. In their morphological details and in their chemical composition the structures formed are, needless to say, widely different in the living system and in its inorganic model, although in certain peculiarities of physico-chemical constitution—especially the cellular and filamentous character of the formations and the part played in both by semipermeable membranes—the precipitation-structures and the living systems show significant resemblances. A precipitation-structure of a definite chemical composition even shows a certain morphological specificity, *i. e.*, the structures formed from zinc are characteristic in their appearance and different from those formed from iron or copper. We may say that under the influence of the metal the ferricyanide of the solution is transformed into structure of a definite kind. And this structure, once formed, becomes the condition of formation of other similar structure.[1]

To complete the resemblance to a growing plant-filament or other proliferating living system, the structures thus formed ought to be capable, after isolation, of forming more structure of the same kind. This is of course not the case with the precipitation-filaments, taken by themselves, since the connection with the metal is essential; but the difference may be regarded as due mainly to incidental conditions. The growing system is in fact not constituted by the filaments *alone*, but by the combination of filaments, metal and solution; the formative process depends upon the interaction of all three. Something analogous may be said to be true of the growing organism; in a spore placed in a

involving electrolysis. This is what takes place in the passage of a current between a metallic surface and a solution; in this case the addition or abstraction of electric charges to or from substances present at the boundary is what forms the essential condition of the electrolysis there taking place. The cell-surface is similarly water-insoluble, and semi-permeability is characteristic; yet it allows the passage of the electric current, although with a somewhat high and variable resistance. The facts of polar stimulation, polar disintegration, etc., indicate that where the current *enters* the cell-surface it produces different chemical effects from where it *leaves*, just as in electrolysis at metallic surfaces.

[1] *Cf.* the description of the mode of formation of precipitation-filaments, *loc. cit.*, pp. 143–144.

culture-medium the structure of the germ, its chemical composition, and the chemical composition of the culture-medium, are all equally essential factors in the resulting growth-process. The main difference is that each living cell, as soon as formed, is capable of acting as a similar center of transformation when transferred to another culture-medium; *i. e.*, *all* of the necessary parts of the proliferating system are multiplied equally; and so far we have been unable to produce any artificial systems having such properties. If we were to succeed in doing so, it is probable that such systems would exhibit a much closer resemblance to living organisms than any of the inorganic models hitherto used for comparisons of the above kind.

Obviously these fundamental resemblances between the two types of system under comparison do not preclude infinite differences in the details of structure, chemical composition, and activity; but I am at present insisting upon the resemblances because of the desirability of determining the *class* to which the organic formative processes belong. The characteristic plasticity and responsiveness of living matter undoubtedly depend upon the fundamental features of its physico-chemical constitution. Starting with living material of this peculiar type of self-regulating structure and chemical composition, the developmental process has in the course of time become so evolved and perfected that it now builds up with unfailing regularity the most complex of organisms from the food and other materials furnished to the germ from the surroundings. But the possibility of this development has depended upon certain general pecularities of physico-chemical constitution present from the beginning in the living formative substance itself; and my aim in the present and preceding papers is to indicate what seem to me the most essential of these peculiarities.

The case of higher organisms presents numerous problems of a more special kind, and most investigators in the field of heredity have given their chief attention to these problems. It seems clear that in these organisms other and more special mechanisms of hereditary coördination and control have been superposed upon the elementary physico-chemical mechanism which conditions the fundamental proliferative activity. The fact that in

higher animals particular cell-structures like chromosomes may become essential regulatory or determinative factors, controlling the detailed character of the developmental proliferation, is in no sense inconsistent with the general point of view here presented. All protoplasmic structures as they originate must influence formative metabolism, as has been so ably pointed out by Child; and there is every evidence that the chromosomes have a peculiarly intimate relation to the distribution of form-determining factors. Recognition of the part played by hormones in development is also consistent with the present view. What we are now attempting, however, is not to define the special mechanisms governing the course of development in the higher metazoa, but to indicate the nature of the more general physico-chemical mechanism, common to all forms of living matter, which forms the basis of its characteristic self-conserving and proliferative properties. Any conception of the essential constitution of living matter must first of all take its constant and fundamental distinguishing peculiarities into account. Once the living material has come into existence, with such properties as these, it may serve as the basis for the development of diversity and complexity of all kinds, as has in fact occurred in the course of evolution.

APPETITES AND AVERSIONS AS CONSTITUENTS OF INSTINCTS.

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GENERAL ACCOUNT OF APPETITE AND AVERSION.

The overt behavior of adult animals occurs largely in rather definite chains and cycles, and it has been held that these are merely chain reflexes. Many years of study of the behavior of animals—studies especially of the blond ring-dove (*Turtur risorius*) and other pigeons—have convinced me that instinctive behavior does not consist of mere chain reflexes; it involves other factors which it is the purpose of this article to describe. I do not deny that innate chain reflexes constitute a considerable part of the instinctive equipment of doves. Indeed, I think it probable that some of the dove's instincts include an element which is even a tropism as described by Loeb. But with few if any exceptions among the instincts of doves, this reflex action constitutes only a part of each instinct in which it is present. Each instinct involves an element of appetite, or aversion, or both.

An appetite (or appetence, if this term may be used with purely behavioristic meaning), so far as externally observable, is a state of agitation which continues so long as a certain stimulus, which may be called the appeted stimulus, is absent. When the appeted stimulus is at length received it stimulates a consummatory reaction, after which the appetitive behavior ceases and is succeeced by a state of relative rest.

An aversion (example 7, p. 100) is a state of agitation which continues so long as a certain stimulus, referred to as the disturbing stimulus, is present; but which ceases, being replaced by a state of relative rest, when that stimulus has ceased to act on the sense-organs.

The state of agitation, in either appetite or aversion, is exhibited externally by increased muscular tension; by static and

phasic contractions of many skeletal and dermal muscles, giving rise to bodily attitudes and gestures which are easily recognized signs or "expressions" of appetite or of aversion; by restlessness; by activity, in extreme cases violent activity; and by "varied effort" (Lloyd Morgan, '96, 7, 122, 154; Stout, '07, 261, 267).

In the theoretically simplest case, which I think we may observe in doves to some extent, these states bring about the appeted situation in a simple mechanical manner. The organism is disturbed, actively moving, in one situation, but quiet and inactive in another; hence it tends to move out of the first situation and to remain in the second, obeying essentially the same law as is seen in the physical laboratory when sand or lycopodium powder on a sounding body leaves the antinodes and comes to rest in the nodes.

But pigeons seldom are guided in so simple a manner. Their behavior involves other factors which must be described in connection with appetite and aversion.

An appetite is accompanied by a certain *readiness to act.* When most fully predetermined, this has the form of a chain reflex. But in the case of most supposedly innate chain reflexes, the reactions of the beginning or middle part of the series are not innate, or not completely innate, but must be learned by trial. The end action of the series, the consummatory action, is always innate. One evidence of this is the fact that in the first[1] manifes-

[1] To see the appetitive nature of an instinct, it is necessary in some cases to observe an individual animal carefully during its first performance of the act in question. But the performance may be so quick that the observer is quite unable to analyze it. Analysis may be aided by preventing the animal from attaining the consummatory situation for a time, so that the appetitive phase is prolonged, as it were magnified. My cripple dove (example 5, p. 99) afforded just this aid to analysis. The literature is full of reports of instinctive behavior which might well be further analyzed. Consider for example the case of the young moorhen cited by Lloyd Morgan ('96, 63) which had never previously dived, but on being suddenly frightened by a puppy, dived like a flash. That act was too quick for us to analyze it. But if we could successfully impede the diving of a young moorhen so as to prolong the phases of the act, I think it probable that we should find an appetite for the consummatory situation (that of being under water) and a restless striving until it is attained; and that some details in the series of actions, details which in a normal dive are very sure to be hit upon by accident, are not innately predetermined. When one sees the first performance of an instinctive act take place very quickly and with apparent perfection, this does not prove that there is an innate chain reflex determining every detail of the act.

tation (also, in some cases, in later performances) of many instincts, the animal begins with an *incipient consummatory action*, although the appeted stimulus, which is the adequate stimulus of that consummatory action, has not yet been received. I speak of an incipient "action" rather than "reaction," because it seems clearly wrong to speak of a "reaction" to a stimulus which has not yet been received. The stimulus in question is obtained only after a course of appetitive, trial-and-error behavior. When at last this stimulus is obtained, the consummatory reaction takes place completely, no longer incipiently. Then the appetitive behavior ceases; in common speech we say the animal is "satisfied."

One may observe all gradations between a true reflex and a mere readiness to act, mere facilitation. Thus, in the dove, a stimulus from food in the crop may cause the parent to vomit the food or to feed it to young: there are all gradations from an immediate crop-reflex, in which the food is vomited upon the ground, through intermediate cases in which the parent is much disturbed by the food in his crop, but appetitively seeks the young and induces them to take the food; to other cases is which the parent is only ready to feed the young if importuned by them; and finally to cases in which the stimulus from the crop does not even cause facilitation, and the parent does not disgorge the food at all, even if importuned by the young.

While an appetite is accompanied by readiness for certain actions, it may be accompanied by a distinct *unreadiness* for certain other actions, and this is an important factor in some forms of behavior. It is altogether probable that this unreadiness is due in some cases to the fact that the activity of certain neurones *inhibits* the activity of certain other neurones. It is now well-known, too, that unreadiness may be due to the condition of the internal secretions. And the mutual exclusion of certain forms of instinctive behavior is inevitable, due to the incompatibility (Washburn, '16) of their motor components.

Unreadiness may be accompanied by aversion, and vice versa; but either of these may occur without the evident presence of the other. An aversion is sometimes accompanied by an innately determined reaction adapted to getting rid of the disturb-

ing stimulus, or—this point is of special interest—by two alternative reactions which are tried and interchanged repeatedly until the disturbing stimulus is got rid of (see example 7, page 100).

The escape from a disturbing situation or the attainment of an appeted one is accomplished, in case of some instincts, far more surely and more rapidly after one or more experiences. In the first performance of an appetitive action, the bird makes a first trial; if this fails to bring the appeted stimulus he remains agitated and active, and makes a second trial, which differs more or less from the first; if this fails to bring the appeted stimulus he remains still active and makes a third trial; and so on until at last the appeted stimulus is received, the consummatory reaction follows, and then the bird comes to rest. In later experience with the same situation, the modes of behavior which were followed immediately by the appeted stimulus and consummatory reaction are repeated; those which were not so followed tend to drop out.

If a young bird be kept experimentally where it cannot obtain the normal stimulus of a certain consummatory reaction, it may vent that reaction upon an abnormal or inadequate stimulus, and show some satisfaction in doing so; but if the bird be allowed at first, or even later, to obtain the normal stimulus, it will be thereafter very unwilling to accept the abnormal stimulus. That this is true of the sex instinct has been shown in a former article (Craig, '14). It is true also of the appetite for a nest. Thus a female dove which has never had a nest, nor material to build one, lays eggs readily on the floor; but a dove that has had long experience with nests will withhold her egg if no nest is obtainable. The male dove similarly, if he has never had a nest, goes through the brooding behavior on the floor; but an experienced male is unwilling to do so, and shows extreme anxiety to find a nest. These examples illustrate the fact that the bird must *learn* to obtain the adequate stimulus for a complete consummatory reaction, and thus to satisfy its own appetites.

There is often a struggle between two appetites, as when a bird hesitates, and it may hesitate for a long time, between going on the nest to incubate and going away to join the flock, eat, etc. By watching the bird one can predict which line of behavior it

will follow, for each appetite is distinguished by its own expressive signs (consisting partly of the incipient consummatory action), and one can see which appetite is gaining control of the organism.

These outward expressions of appetite are signs of physiological states which are but little known. Since my own observations have been on external behavior only, I say little about the internal states. They are probably exceedingly complex and numerous and similar to the physiological states which in the human organism are concomitants of appetites,[1] emotions, desires. They doubtless include stimulations from interoceptors and proprioceptors; perhaps automatic action of nerve centers; perhaps readiness or unreadiness of neurones to conduct. It is known that some of the periodic appetites are coincident with profound physiological changes. Thus Gerhartz ('14) found that during the incubation period in the domestic fowl the metabolism of the body as a whole is at a low ebb. In some cases a stimulus from the environment is the immediate excitant of an appetite; especially, stimulation of a distance-receptor may arouse appetite for a contact stimulus, as when the sight of food arouses appetite for the taste of it. But probably in every case appetite is dependent upon physiological factors. And in many cases the rise of appetite is due to internal causes which are highly independent of environmental conditions, and even extremely resistant to environmental interference.

Appetitive behavior in vertebrates is evidently a higher development of what Jennings ('06, p. 309) calls the positive reaction in lower organisms; aversive behavior in vertebrates corresponds to what Jennings (p. 301) calls negative reactions.

The attempt to distinguish between instinct and appetite, as in Baldwin's Dictionary ('01), is not justified by the facts of behavior. Baldwin says : "Appetite is distinguished from instinct in that it shows itself at first in connection with the life of the organism itself, and does not wait for an external stimulus, but appears and craves satisfaction." These characteristics,

[1] Hunger furnishes a typical case of appetitive behavior (Carlson, '16; Ellis '10, 198–199). Carlson makes a distinction between hunger and appetite. The distinction he finds is certainly real, but the use of words is unfortunate, for hunger is clearly one kind of appetite.

here ascribed to appetite, are the very ones which I have observed in the instinctive behavior of pigeons. The instincts of pigeons satisfy Baldwin's further description of appetite in that each appears first as a "state of vague unrest" involving especially "the organs by which the gratification is to be secured"; and "a complex state of tension of all the motor . . . elements whenever the appetite is aroused either (*a*) by the direct organic condition of need, or (*b*) indirectly through the presence or memory of the object." This last point is illustrated, *e. g.*, by doves learning to drink (example 1, page 97), in whom the sight of the water-dish at a distance aroused the drinking actions by associative memory. I have observed appetitive behavior as Baldwin describes it in nearly all the instinctive activities of doves, and I think that sufficient observation will reveal it in all their instincts.

The most thorough attempt to distinguish instincts from appetites and to show the logical consequences of such distinction, in all the literature to which I have access, is in an old article by Professor Bowen ('46). This article is still worth study, to suggest the conclusions to which one is logically led if he denies that instincts contain any element of appetite. These conclusions, taken almost literally from Bowen, may be summarized as follows: (1) (P. 95) "If the name of instinct be denied to these original and simple preferences [appetites] and aversions, there will appear good reason to doubt whether man is ever governed by instinct, whether all his actions are not reducible to passion, appetite, and reason." (P. 115) The "passions" of man can not be concomitants of instinct. (2) (P. 117) "Instinct is not a free and conscious power of the animal itself. It is, if we may so speak, a foreign agency, which enters not into the individuality of the brute." (P. 118) Instinct "has no effect on the rest of their conduct, which is governed by their own individuality." (3) Bowen contends with logical consistency that if instinct contains no appetitive factor, the ends toward which instincts work, as seen by an observer, are not ends for the agent; that therefore the agent has no power to make the instinctive behavior more effective. In short, instinctive behavior is not susceptible of improvement by intelligence. (4)

Bowen concludes that the intellect and the "passions" of man are not products of evolution. (5) It may be added that even Bowen, strive as he did to separate appetite from instinct, was compelled to admit that the attempt at such separation leads one into difficulties and disputed cases. In contravention of Bowen's conclusions I contend: (1) That much of human behavior is instinctive. (2) That Bowen's description of instinct as "a foreign agency, which enters not into the individuality" is true of reflex action, such as coughing or sneezing, but is not true of instinctive behavior, which is extremely different from such mere reflexes. (For a fuller statement on this point, see below, page 106. See also Hobhouse, '15, 98–99.) (3) That, of the useful results toward which instincts tend, some, not all, are ends for the agent. For they are the objects of appetites, and the animal strives and learns to attain them. (4) That human conative behavior evolved from the instinctive appetitive behavior of lower animals.

In another article I hope to publish soon a further discussion of the literature.

Examples.

1. The case of doves learning to drink, as described in detail in a former article (Craig, '12), illustrates appetite. The observed appetitive behavior was aroused by stimulation of distance-receptors, such as the sight of the water-dish being brought to the cage, and of the man bringing it; these acted as appetizers. Each dove, as soon as it had learned to associate such stimuli with the drinking situation, responded to these stimuli by making drinking movements (incipient consummatory action) at once without going to the water dish. The first drinking movements failing to bring water, the dove repeated these movements again and again, sometimes walking a few steps, sometimes turning round, until after many trials and many errors it did get its bill into the water, received the stimulus from water in the mouth (appeted stimulus), whereupon the drinking movements (consummatory reaction) were made not incipiently but completely, the water being swallowed, after which the bird rested and appeared satisfied.

2. A good example of appetitive behavior is seen in the way in

which a young male dove locates a nesting site for the first time. The first thing the observer sees is that the dove, while standing on his perch, spontaneously assumes the nest-calling attitude, his body tilted forward, head down, as if his neck and breast were already touching the hollow of a nest (incipient consummatory action), and in this attitude he sounds the nest-call. But he shows dissatisfaction, as if the bare perch were not a comfortable situation for this nest-dedicating attitude. He shifts about until he finds a corner which more or less fits his body while in the tilted posture; he is seldom satisfied with his first corner, but tries another and another. If now an appropriate nest-box or a ready-made nest is put into his cage, this inexperienced dove does not recognize it as a nest, but sooner or later he tries it, as he has tried all other places, for nest-calling, and in such trial the nest evidently gives him a strong and satisfying stimulation (the appeted stimulus) which no other situation has given him. In the nest his attitude becomes extreme; he abandons himself to an orgy of nest-calling (complete consummatory action), turning now this way and now that in the hollow, palpating the straws with his feet, wings, breast, neck, and beak, and rioting in the wealth of new, luxurious stimuli. He no longer wanders restlessly in search of new nesting situations, but remains satisfied with his present highly stimulating nest.

3. Fetching straws to the nest is apparently due to an appetite for building them into the nest. The dove has an innate tendency to pick up straws, and an innate tendency to build them into the nest (consummatory reaction); but it has apparently no innate tendency to carry a straw to the nest, no innate "chain" of reflexes. When an experienced bird finds a straw he seizes it repeatedly and toys with it, sometimes making movements resembling those by which he would build the straw into the nest. He seems thus to get up an appetite for building the straw in, and when this appetite is sufficiently aroused he flies to the nest, guided by associative memory, and performs the consummatory reaction completely. A young female, no. 70, which I observed picking up a straw for the first (?) time, on her 54th day, showed the lack of a "chain reflex." For she continued toying with the straw an excessively long time, not carrying it at all,

though she happened to be very near the nest. This was the more remarkable as she had a well-formed habit of going to the nest on all occasions. At length she did go to the nest with her straw, and made well-ordered movements to build it in.

4. The male and the female dove take regular turns in sitting on the eggs. The male is seized by the appetite for brooding about 8 or 9 A. M., and the female about 5 P. M., the state evidently being brought on in each case by physiological causes which are part of the daily physiological rhythm. When either one, *e. g.*, the female, comes to the side of the nest prepared to enter and sit, she already has somewhat the attitude of the sitting bird, the body sunk down on the legs and the feathers fluffed out (incipient consummatory action). If her sitting appetite be thwarted, as by her mate refusing to budge from his position, she shows restlessness and makes intelligent efforts to obtain possession of the nest. When at last her mate yields his place, she steps exultingly into the center of the nest and settles herself on the eggs with many movements indicative of satisfying emotion (complete consummatory reaction).

A broody hen of course illustrates the same principle.

5. It is an interesting fact, exhibited in a variety of instincts, that a young bird may make feints of performing actions which it has never yet performed. Thus the young dove makes feints of flying before it has ever flown. This was illustrated in a peculiarly instructive manner by one of my young doves, no. 46, which developed cripple wings and was unable to fly. When placed in a box with sides 3½ inches high it was just able to jump on the edge. Nevertheless, when its roosting instinct developed, it endeavored strenuously every evening to fly to the perch which was some inches above its head. It looked at the perch and aimed at it with perfect definiteness, opening its wings and making feints of flying. In the evolution of birds, there can be no doubt, flying developed gradually from jumping. The new movements of flying were gradually intercalated into the interval between the initial action, leaping from the ground, and the final action, landing again upon the feet. The young dove to this day shows *first* the incipient end action, aiming at the perch to be alighted on, and only after it has launched itself

toward this end situation does the "chain" of flight reactions take place.

6. In the pigeons the order of activities culminating in the sexual act is, first display, second billing, third copulation, with numerous details each finding a place in the succession. Yet the sexual tendency is mainfestly present from the beginning of the "chain," and the preliminary steps are directed, with much guidance by experience, toward securing the stimulation required for discharging the sexual reflex. In absence of the normal stimulus to the consummatory reaction, the instinct manifests itself in marked appetitive behavior, and, especially in inexperienced birds (Craig, '14), in those imperfect consummatory reactions known as perversions and auto-erotic phenomena. The behavior of the sexual appetite is now so well known that it may be cited as the type of appetitive behavior; and to readers who are familiar with modern analyses of the sex instinct I may make my whole article clearest by saying that all the appetitive mechanisms I have mentioned, and I believe all the instincts of the dove, behave in the same manner as that of sex, in regard to appetitive manifestations and anticipation of the consummatory reaction.

7. I shall take space to describe only one example of aversion—the so-called jealousy of the male dove, which is manifested especially in the early days of the brood cycle before the eggs are laid. At this time the male has an aversion to seeing his mate in proximity to any other dove. The sight of another dove near his mate is an "original annoyer" (Thorndike, '13, Chap. IX.). If the male sees another dove near his mate, he follows *either of two* courses of action; namely, (*a*) attacking the intruder, with real pugnacity; (*b*) driving his mate, gently, not pugnaciously, away from the intruder. When he has succeeded either in conquering the stranger and getting rid of him, or in driving his mate away from the stranger, so that he has got rid of the disturbing sight of another dove in presence of his mate, his agitation ceases. If we prevent him from being successful with either of these methods, as, by confining the pair of doves in one cage and the third dove in plain sight in a contiguous cage, then he will continue indefinitely to try both methods. If we leave all three

doves free in one pen, the mated male will try the mettle of the intruder and conquer him if he can; if he fails, he will turn all his energies into an effort to drive his mate away from the intruder. Or if in former experiences he has learned to gage this individual intruder, if he conquered him before he will promptly attack him now, but if defeated by him before he will now choose the alternative of driving his mate away. In sum, the instinctive aversion impels the dove to thoroughly intelligent efforts to get rid of the disturbing situation.

8. In some cases the seeking of a certain situation involves both appetences and aversions in considerable number. Thus, when the day draws to a close, each dove seeks as its roosting-place a perch that is high up, with free space both below it and above it, with no enemies near, with friendly companions by its side, but these companions not too close, not touching (except in certain cases of mate, nest-mate, or parent). The endeavor to achieve this complex situation, to secure the appeted stimuli and to avoid the disturbing ones, keeps the birds busy every evening, often for an hour or more.

Cycles.

Instinctive activity runs in cycles. The type cycle, as it were a composite photograph representing all such cycles, would show four phases as follows.

Phase I.—Absence of a certain stimulus. Physiological state of appetite for that stimulus. Restlessness, varied movements, effort, search. Incipient consummatory action.

Phase II.—Reception of the appeted stimulus. Consummatory reaction in response to that stimulus. State of satisfaction. No restlessness nor search.

Phase III.—Surfeit of the said stimulus, which has now become a disturbing stimulus. State of aversion. Restlessness, trial, effort, directed toward getting rid of the stimulus.

Phase IV.—Freedom from the said stimulus. Physiological state of rest. Inactivity of the tendencies which were active in Phases I., II., III.

Some forms of behavior show all four phases clearly. The following are examples.

Sex.—(Phase I.) The dove, either the male or the female, shows sexual appetite and invites the mate to sexual activity. Gradually they lead up to (Phase II.) the consummatory sexual act. (Phase III.) After the sexual act, in some cases one bird shows marked aversion, *e. g.*, by striking at the mate. Either the male or the female may show aversion. In some species, signs of aversion after the sexual act seem to be a normal and regular occurrence. In other species they are shown only by a bird whose mate, having failed of satisfaction, invites to further sexual activity. (Phase IV.) The pair usually become sexually indifferent for a considerable time after each copulation.

Brooding.—(Phase I.) The dove shows the brooding appetence, goes to the nest, and, if need be, struggles to obtain possession of it. (Phase II.) It sits throughout its customary perood, during which it often resists efforts of the mate to relieve it. (Phase III.) At the end of this period, in contrast, it comes off at a slight sign from the mate, runs about, flaps its wings, and thus shows its joy in being off. This may be interpreted as a sort of mild aversion for the nest. (Phase IV.) It goes away and becomes temporarily indifferent to the nest.

In other cases, one or other of the phases is not clearly present, so that there are various sorts of incomplete cycles, such as the following.

(*a*) When the bird shows appetitive behavior but fails to obtain the appeted stimulus, the appetite sometimes disappears, due to fatigue or to drainage of energy into other channels; in which case, Phase II. is not attained.

But many instinctive appetites are so persistent that if they do not attain the normal appeted stimulus they make connection with some abnormal stimulus (see page 94); to this the consummatory reaction takes place, the tension of the appetite is relieved, its energy discharged, and the organism shows satisfaction. This is of course *compensation*, in the sense in which that word is used in psychiatry. But the abnormal stimulus is usually inadequate or incomplete, the relief or discharge is imperfect, the satisfaction is marred by the fact that some of the constituent elements of the appetite, failing to receive their appeted stimuli, are still in Phase I. and abnormally active, while at the same time other elements have already reached Phase III., aversion.

(*b*) Some forms of behavior consist of appetite and satisfaction which are not, in ordinary cases, followed by any distinct aversion. For example, the drinking cycle shows clearly: (Phase I.) appetite for water; (Phase II.) the drinking reaction, with expression of satisfaction; (Phase IV.) indifference. The dove when it finishes drinking shows no distinct sign of aversion (Phase III.) except withdrawing the bill from the water. But if the observer takes this dove then gently in the hand and re-submerges its bill in the water, it shows marked aversion, struggling to withdraw the bill and to shake the water out of it.

(*c*) On the other hand it may seem that there are some forms of behavior, *e. g.*, fear, in which Phases I. and II. are lacking; that there is no appetite for the fear stimuli and no satisfaction in them; that when the slightest of these stimuli is received it at once arouses (Phase III.) aversive behavior. Yet it is an interesting fact that even in these cases a slight degree of appetite and satisfaction may be present. Children seek and enjoy a little fear. A dove, when it hears the alarm cry from other doves, at once endeavors to see the alarming object. Even pain is (in man) to some degree, sought and enjoyed.

In actual life the cycles and phases of cycles are multiplied and overlapped in very complex ways.

For example, when a certain satisfaction has been attained, this, instead of leading at once to a state of surfeit and aversion, may lead to further appetite, which leads to a second satisfaction, and so on. Thus Phase I. and Phase II. continue to alternate, constituting a "circular reaction" (Baldwin). I have seen a pair of house sparrows copulate thirteen times in immediate succession, and know by the sound of their voices that I did not see the beginning of the series. In many cases such circular reaction serves to rouse the organism to a high state of appetite and readiness for action.

Smaller cycles are superposed upon larger ones. For example, when a female bird is building a nest, so long as she is in the nest she is in a certain nest-building attitude, a high state of satisfaction, which constitutes the consummatory reaction (Phase II.) of a large cycle. But each time she reaches for a straw, seizes it, and tucks it into the nest, she exhibits thus a little cycle containing a little appetence followed by its own satisfaction.

The time occupied by a cycle varies extremely, from cycles measured in seconds to those that occupy a year or even longer. The relative duration of the phases also is extremely variable. In some cases the appeted situation is attained without delay, and Phase I. thus passes so rapidly as to be overlooked by the observer. In other cases the bird strives hard to overcome great obstacles which stand in the way of the attainment of the appeted stimulus, consequently Phase I. is of long duration. Phase II. may last, in the case of drinking, about one second; in the case of incubation, about three weeks.

It should be stated, too, that the phases are not sharply separated; each passes more or less gradually into the next. Thus, from Phase IV. of one cycle in a series to Phase I. of the succeeding cycle, there is often a gradual rise of appetite; active search for satisfaction does not commence until a certain intensity of appetite is attained. This is what is known in pedagogical literature as "warming up." This gradual rise of the energy of appetite is followed (Phase II.–III., or II.–IV.) by its sudden or gradual discharge. This rise and discharge are named by Ellis ('03), in the case of the sex instinct, "tumescence" and "detumescence." They are important phases in the psychology of art, in which sphere they are named by Hirn ('00) "enhancement" and "relief." The discharge (Phase II.) is also exemplified in "catharsis" in art and in psychiatry.

The cycles in the behavior of birds are fundamentally the same phenomenon as the cycles in human behavior. Human cycles are enriched by an intelligence far surpassing that of doves, but this is a difference of degree only. If the dove's cycles are determined largely by instinct, habit, physiological conditions, and not intelligence, so are some human cycles, as those of sleeping, eating, drinking, sex. F. H. Herrick ('10, 83) emphasizes the fact that a bird may scamp one cycle in order to begin another. Thus, birds may abandon young which are not yet weaned, because their appetence for a new brood has set in. But the same principle works, though not quite so crudely, in human life: as in the case of a mother who grows indifferent or even somewhat hostile toward her older children each time a new child is born. Herrick emphasizes also the fact that when anything disturbs

the bird in the progress of a cycle, she very often gives up that cycle and begins a new one. Thus, a cedarbird who has just completed her nest one day finds a man examining it; she forthwith abandons that nest and begins to build another. But, again, the same phenomenon appears in human behavior. A man begins to build a house; when he has progressed far with the building he meets some horrible experience in it which "turns him against" it, and nothing will induce him to proceed with that house; he abandons it and begins to build elsewhere. The cedarbird has had a, to her, horrible experience which has turned her against her nest; that nest has lost its *value* for her; the sight of it now, instead of arousing her appetence, arouses aversion.

C. J. Herrick ('15, p. 61) says that many of these cyclical activities of birds are "simply complex chain reflexes." The reason he gives for this statement is that "each step in the cycle is a necessary antecedent to the next, and if the series is interrupted it is often necessary for the birds to go back to the beginning of the cycle. They cannot make an intelligent adjustment midway of the series." But all this, in some degree, is true of the behavior of human beings toward their mates, their nests, and their young. This has been illustrated in the preceding paragraph, and a few illustrations are here added. As to mates: When the cordial relation between a husband and wife is, by some mischance, broken, the pair may make an "intelligent adjustment" if the difficulty is not too great. But birds also make such adjustments constantly, when the difficulty is not too great. And with human beings, as with birds, the difficulty may be insurmountable; in which case, the husband and wife separate for a week, a month, or a year, after which period of rest (Phase IV.), they can commence a new cycle with Phase I., courtship. As to their nests: The fact of homesickness proves that the behavior of a human being toward his or her home runs in a series which conforms to Herrick's statements. As to behavior toward the young: The inability of human parents to make "an intelligent adjustment midway of the series" is shown by the fact that they cannot arouse the fullest degree of parental behavior toward an adopted child unless they adopt the child in its infancy. These facts do not prove that the human behavior

in question consists of mere chain reflexes. Neither do the similar facts as to avian cycles prove that the avian behavior consists of mere chain reflexes.

The birds in their cycles exhibit attention (using this and all the following terms in a strictly behavioristic sense), intelligence, memory, intensely emotional behavior, conflict of tendencies, hesitation, deliberation (of course an elementary sort of deliberation), rise, maintenance, and decline of appetences, behavior conformable to certain laws of valuation. All these forms of behavior function in bringing about the consummatory situations of the cycles. Thus the instinctive behavior of birds, so far from consisting of mere chain-reflexes, and having no relation to "individuality" (Bowen, vide ut supra, p. 97), is in reality very highly integrated, and is the very core of the bird's individuality.

All human behavior runs in cycles which are of the same fundamental character as the cycles of avian behavior. These appear in consciousness as cycles of attention, of feeling, and of valuation.

This description is true not only of our behavior toward objects specifically sought by instinct, such as food, mate, and young, but also of our behavior toward the objects of our highest and most sophisticated impulses. Consider, for example, the course of a music-lover's feelings and attention in the case of a symphony concert. Before the concert, if his internal state is favorable (Phase I.), he is all eagerness, desire, interest. He goes to the concert-hall, chooses a good seat for hearing, and in every way shows appetitive behavior. (Phase II.) The music begins, he pays close attention, and feels satisfaction. (Phase III.) If the concert continues too long, he is surfeited, his pleasure diminishes, he even feels some unpleasantness, and his attention turns away, which is of course a form of aversion. (Phase IV.) When the music at length ceases he feels restfulness, relief, and his attention goes elsewhere. This cycle of the whole concert is overlaid by a complex system of epicycles, each extending through one symphony, one movement, or a smaller division, down to the measure and the beat. This is only one illustration of the fact that the entire behavior of the human being is, like that of the bird, a vast system of cycles and epicycles, the longest cycle extending through life, the shortest ones being measured

in seconds. This view helps us to understand the laws of attention; for example, the law that attention cannot be held continuously upon a faint, simple stimulus. For as soon as such a stimulus is brought to maximum clearness, which constitutes the consummatory situation, the appetite for it is quickly discharged and its cycle comes to an end. This familiar fact shows that we, like the birds, are but little able to alter the course of our behavior cycles.

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FURTHER STUDIES ON THE GEOTROPISM OF PARAMECIUM CAUDATUM.[1]

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(With two figures.)

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I. INTRODUCTORY.

As evidence of the existence of proper mechanical conditions in protoplasm as a basis for the "statocyst theory" of geotropism in *Paramecium*, Lyon states: "The animals were strongly centrifuged for several minutes in the hæmatocrit attachment. Microscopic examination showed that certian dark granules originally distributed were now aggregated in one end, usually the anterior. It is thus seen that differences in specific gravity exist in the protoplasm of this animal."[2] In previous experiments in which he attempted to test Lyon's results, the writer "could obtain nothing definite"; and the tentative suggestion was made that "immediately after centrifuging a capillary tube containing *Paramecia*, and in which the latter can not turn around, we may stain them with some dyes and determine the effects

[1] From the Physiology Laboratory of the University of Minnesota.

[2] Lyon, E. P., 1905, "On the Theory of Geotropism in *Paramecium*, *Am. Jour. Physiol.*, Vol. 14, p. 430.

of the centrifugal force upon the protoplasmic materials of the organisms."[1]

While the writer was conducting experiments with a vital stain method in the physiological laboratory of the University of Minnesota, and after he had obtained some valuable results, he learned that McClendon had already published satisfactory results in 1909.[2] The writer found that McClendon's method showed better results than those obtained with vital stains and has, therefore, adopted that method for this investigation, although not able to agree with all of McClendon's interpretations.

This paper attempts to show that *Paramecium caudatum* contains protoplasmic substances of different specific gravities, and subjects to further experimental examination the various phenomena of geotropism in the animal, described by various investigators. The experimental work was done during the academic year of 1913–1914, while the writer was holding a Shevlin Fellowship in the medical school of the University of Minnesota.

II. Material.

A dense culture raised in hay infusion from a single individual was used.

III. Experimental.

1. *The Specific Gravity of Paramecium caudatum.*

As to the difference of the specific gravity of the *Paramecium* between Lyon's 1.048 or 1.049 and the writer's 1.037 or 1.037 ± 0.003, the latter suggested[3] a possible source of error in Lyon's experiments. The writer this time examined the subject more carefully.

He prepared a gum-arabic solution using distilled water, as in the case of his previous experiments. He dialyzed the neutral solution through parchment paper. The specific gravity of the solution so prepared was determined by means of a U-shaped picnometer, as 1.0426.

[1] Kanda, Sakyo, 1914, "On the Geotropism of *Paramecium* and *Spirostonum*," Biol. Bull., Vol. 26, p. 22.

[2] McClendon, J. F., 1909, "Protozoan Studies," *Jour. Ex. Zool.*, Vol. 6.

[3] Kanda, Sakyo, *loc. cit.*

Then the writer made many small tubes for hæmatocrit attachment, of about 4.7 cm. long and 0.3 cm. in diameter, inside measure. One end of each tube was sealed. One of these tubes was filled with the gum-arabic solution mentioned above and the number of drops which were put in the tube was counted. Now, to the number of the drops of the solution, one drop of water was added. Thus the difference of the specific gravity of the solution before and after the addition of one drop of the latter to the number of drops of the former could be estimated. The writer thus found that the addition of only one small drop of water to the gum-arabic solution of the known specific gravity, that is, 1.0426, lowered the latter to 1.0415. And when another drop of water was added to this, it became about 1.0404. The addition of one drop of water, therefore, lowered the specific gravity of gum-arabic solution about 0.0011.

A pair of tubes was thus prepared with a definite number of drops of the gum-arabic solution of the specific gravity 1.0415. This pair was centrifuged as usual. Then on the top of the solution of one tube, one drop, and on the other, two drops of water containing dense *Paramecia* were added. The tubes were again centrifuged for two minutes with a speed of about 7,300 revolutions per minute. The results were determined by means of a magnifying glass immediately after the centrifugalization. All the procedure was the same as the writer described in his previous paper. Thus the nearest possible density of the animal was obtained:

TABLE I.

I.	Tube 1. Dens. of gum-arab. sol. 1.0404. All stay at upper part. Tube 2. Dens. of gum-arab. sol. 1.0393. Majority stay near middle.	
II.	Tube 1. Dens. of gum-arab. sol. 1.0382. Many stay at middle. Tube 2. Dens. of gum-arab. sol. 1.0371. A few go to bottom.	

This was carefully tried with different cultures and no practical difference was found. The writer, therefore, concludes that the specific gravity of *Paramecium caudatum* is between 1.0382 and 1.0393.

In comparison with the writer's previous results, that is, 1.037 or 1.037 ± 0.003, the difference is not great. In his previous experiments, however, he was not critical enough to test the lowering of the specific gravity of the gum-arabic solution after one or two drops of water containing *Paramecia* were added to the tubes. The writer, therefore, considers that the present result is more correct than the previous one.

A more accurate estimation could possibly be made by having the animals before adding them to the centrifuge tube in gum solution of a density only a little less than that of the animals. The lowering of specific gravity by mixture would thus be lessened. Nevertheless, the writer believes that the assumption of 1.039 as the specific gravity of *Paramecium caudatum* is close enough for biological purposes.

2. *The Effects of Centrifugal Force on the Protoplasm of Paramecium caudatum.*

The specific gravity of the animal being known as about 1.039, a gum-arabic solution of higher specific gravity, that is, about 1.1, was prepared. If *Paramecia* are strongly centrifuged in such a solution, they all should be suspended in the solution. And the heavier end of the animals should, also, be passively thrown away from the axis of the centrifuge. Now the writer put a certain number of drops of the gum-arabic solution in a pair of hæmatocrit tubes already described, and on the top of each, one drop of water containing dense *Paramecia* was added. The solution and the water in the tubes were well mixed by means of a fine glass needle. The tubes were centrifuged for fifteen minutes at a velocity of 108.3 revolutions per second and with radius of 2.5 cm. to 7 cm.

The paramecia with some of the gum-arabic solution, after being centrifuged, were sucked out in a capillary tube and were killed in 1 per cent. chromic acid, being left in the solution for about one minute. Then they were stained by McClendon's method, "in Biondi's methyl green, orange G and acid fuchsin mixture with a little less fuchsin and of about one fourth saturated strength"[1] for about four minutes, dehydrated and mounted.

[1] McClendon, J. F., *loc. cit.*

The microscopic examination of the preparations showed that the darker and heavier substances and crystal-like materials lay in the extreme anterior end of the animal which was thrown away from the axis of the centrifuge. Next to these came the micro-

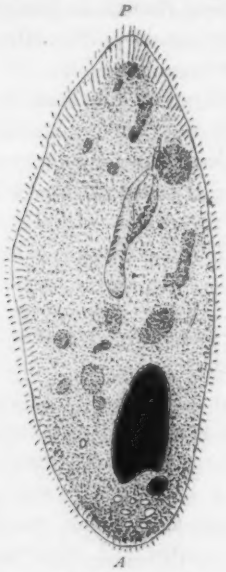

FIG. 1.

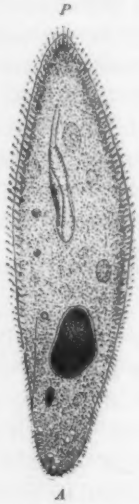

FIG. 2.

FIGS. 1 and 2. Camera drawings of *Paramecium caudatum*. *A*, anterior end; *P*, posterior end.

nucleus and then the macro-nucleus. The chromatin which was stained green, seemed to have been precipitating to the outer nuclear wall, since this part of the nucleus was stained much darker than the inner, as Figs. 1 and 2 have shown. The "plasmosomes" and cell granules were stained orange, but the cilia did not show very distinctly. The writer thus found in confirmation of Lyon's and McClendon's results that *Paramecium* contains protoplasmic materials of differing specific gravities. Figs. 1 and 2 show specimens drawn with the aid of a camera attachment.

That the anterior end of the animal, when centrifuged, was thrown away from the axis of the centrifuge presumably on ac-

count of its heavier weight, was originally demonstrated by Lyon. This was the strongest in his experiments to cast aside the "mechanical theory." The writer supported him by furnishing results obtained by improved methods to meet Harper's criticism.[1] Harper insisted that in strong centrifugalization, "the same effect is produced at the outset as by mechanical agitation, *i. e.*, the reaction changes to positive." In other words Harper claims that the head is turned outward by a positive response on the part of the organism, and not passively, as claimed by Lyon. The writer showed in his previous paper that Harper could not be right.

However, McClendon thinks that "the geotropic reaction" of the animal "may be strong enough to turn the anterior end in the opposite direction" toward the axis of the centrifuge against the centrifugal force. This needs a serious examination. If the anterior end of the animal is considered "heavier" than the posterior, as Lyon and Kanda hold, it is hardly conceivable that such a small animal as *Paramecium* could orient itself with the heavier end toward the axis of the centrifuge against the force McClendon used. On the other hand, if the posterior end is regarded "heavier" than the anterior, as Verworn[2] and Harper hold, one meets the same difficulty to conceive how the animal can orient itself with the "lighter" anterior end away from the axis of the centrifuge against the much greater force used by Lyon and the writer. McClendon admits that "this is usually the initial orientation," but seems to think the animals may occasionally turn around later by an active reaction. The writer must confess that he cannot conceive its possibility. He found one individual among several dozen examined which was thrown with the posterior end away from the axis of the centrifuge. He considers, however, that this was a mere accident, the animal being oriented in that direction at the beginning of centrifugalization and forced into the capillary before passive turning could occur. McClendon's case was also probably exceptional and moreover had been centrifuged twenty-four hours, during which

[1] Harper, E. H., 1911, "The Geotropism of *Paramecium*," *Jour. Morph.*, Vol. 22, p. 998.

[2] Verworn, Max, 1889, "Psycho-Physiol. Protisten-Studien," Jena: Gustav Fischer, p. 121.

admitted changes in specific gravity had occurred, due to loss of water. If this loss were chiefly from the posterior part, the changed orientation would be explained as a passive instead of an active process. The writer, therefore, holds still that these animals, when strongly centrifuged, are *passively* thrown with their anterior ends away from the axis of the centrifuge. Therefore, the anterior end is heavier. The negatively geotropic orientation in the normal animals is, therefore, an active process and the mechanical theory is not correct.

McClendon "found the time elapsing before the return of the negatively geotropic reaction to roughly correspond to the time required for the return of the nuclei to their normal position." The writer also found that this was the case. According to McClendon, "this might indicate that nuclei in normal position acted as statoliths." It is hard to decide for or against this for at the time of "the return of the nuclei to their normal position," other heavy substances, "phosphate crystals," for instance, which were precipitated in the extreme anterior end of the animal seem, also, to distribute in their original position. In other words, about at the time of return of the nuclei to their normal position, all the protoplasmic substances of the animal, which were disturbed by the centrifugal force from their original distribution, recover from the disturbance. The result is that the animal resumes the negatively geotropic reaction. Such consideration may suggest that the whole organism in normal conditions acts as a "statecyst," and all the heavy substances as "statoliths," not merely the nuclei. Here lies the significance of Lyon's conception that "for internal stimulation the relation of the parts of the cell to each other must be changed in some way by gravity. Stresses or pulls which occur when the organism is in one position with respect to the vertical, must be changed in another position." For the present we cannot decide in favor of any particular organ or constituent of the cell as the basis of the reaction.

3. Does temperature reverse the negative geotropism of *Paramecium caudatum?*

From his observations on the effects of raising and lowering temperature on the geotropism of this animal, Sosnowski con-

cludes: "Es hat sich dabei herausgestellt, dass durch Einwirkung höher Temperaturen . . . bei vielen Culturen vorübergehend positiver Geotropismus hervorgerufen werden kann." And "durch die Temperaturerniederingung (bis + 2°) konnte ich auch bei den sehr dazu geeigneten Aquarien keinen positiven Geotropismus hervorufen."[1] On the contrary, Moore[2] states: "Several tubes were left for three hours in a thermostat at a temperature of 26°–28° C. The *Paramecia* collected at the top in dense clusters." And "tube *B* was placed in a larger tube filled with water and surrounded by a mixture of ice and salt, the temperature being kept as nearly as possible at 1° C. In ten minutes the *Paramecia* in tube *B* were massed at the bottom, and two hours later were still massed there."

Sosnowski and Moore, however, gave no consideration to the phenomena of the convection currents which would invariably occur, when water was cooled or heated. That water currents would produce effects on the geotropism of the animal was mentioned by the writer in his previous paper. The animals are rheotropic. Moreover, the animals being so small cannot resist any strong current. Consequently they would be passively carried by the current, when it was strong. There was, therefore, needed a special device to minimize these effects as much as possible.

Dewar vacuum tubes served to some extent for this purpose. The tubes were 3.2 cm. in diameter and 27 cm. long inside measure. Three tubes, one for control and the others for experiments, cold and warm, were used for one series of experiments. It was noted that warm water of 30° or 35° C., which was placed in one of the tubes, with a rubber stopper, cooled off about 2° C. after one hour, and water of 1° to 4° C. in the other became warmer 2° C. in about half an hour. Temperature of the control, that was room temperature, was about 20° C. and fairly constant. The dense culture of animals that were thoroughly washed was cooled or warmed as desired. And a few drops of the culture so

[1] Sosnowski, J., 1899, "Untersuchungen über die Veränderungen der Geotropismus bei *Paramecium aurelia*," *Bull. Intern. d. l'Acad. d. Sci. d. Cracovie*, S. 134.

[2] Moore, Anne, 1903, "Some Facts concerning the Geotropic Gathering of *Paramecium*," *Am. Jour. Physiol.*, Vol. 9, pp. 239 and 240.

prepared were transferred by means of a pipette into the Dewar tube with special care to make the least current. The tube was closed with a rubber stopper. The results based on many experiments are given in Table I.

TABLE I.

EFFECTS OF TEMPERATURE (?).

Time.	Cold.	Control.	Warm.
	1°–2° C	20° C.	20°–30° C.
Immediately after.	Very sluggish and hardly active.	Majority swim downward.	Abnormally active and majority swim down.
5–10 m. later.....	Not very active and scattered all over.	Many at the lower part.	Many at the lower part.
20–30 m. later....	A little more at the lower part and becoming somewhat active. The temp. about 4° C.	Many at the upper part.	A little more at the lower part. The temp. about 29° C.

Cooling water to 3°, 4°, or 5° C. and warming to 35° C. were also tried. At a temperature 5° C., the animals were somewhat active, and showed a tendency to swim downward. Another series of experiments was also carried out in an incubator and in an ice box. Each experiment lasted three days. The temperature in the incubator ranged from 30° C. to 34° C. In one series of the experiments, the animals so treated in the incubator were "positively geotropic"? That is to say, a majority of the animals always staid at the bottom of the tube, as Sosnowski observed. But in others, the results were very irregular and variable. Experiments in the ice box were not satisfactory, because it was very hard to keep the temperature constantly low.

The writer found that the animals always swam downward, whenever transferred, no matter how carefully it was done. This occurred invariably even in water of the same temperature as the culture, when the animals were transferred into it. It seems to the writer, therefore, to be possible that this reaction was mistaken as reversibility of geotropism of the animals by temperature. That the animals swim downward, *i. e.*, their negative geotropism is reversed, whenever transferred, would suggest

that mechanical agitation of transferring may be responsible for it. Sosnowski also suggested this factor in one case, "die Temperaturerhöhung und Erschütterung sich zu summieren scheinen" The reversibility of the negative geotropism of the animals by mechanical "shock" or agitation is a well-known fact. The water current may also help it to some extent.

From these results the writer concludes that so-called reversibility of the negative geotropism of *Paramecium* by temperature is extremely doubtful. Especially in such cold water as near 1° C., the animal is almost, if not thoroughly anesthetized. In consequence, it sinks by its weight, though Moore thinks it is "reversed."

4. Do chemicals reverse the negative geotropism of *Paramecium caudatum?*

According to Sosnowski, "durch Zusatz geringerer Mengen von Säuren oder Alkalien (1 bis 3 c.c. Salzsäure oder Natronlauge von 0.5 per cent. auf 20 c.c. Culturflüssigkeit) kann man verubergehenden positiven Geotropismus hervorufen." Moore also states that "in $n/16$ NaCl, they went to the bottom almost immediately, but shortly after died." In a solution of calcium chloride isosmotic with $n/32$, "they went immediately to the bottom."

The writer attempted to test these statements of Sosnowski and Moore. First of all, he thoroughly washed the animals in boiled then cooled tap water. Chemical solutions from the strongest to the weakest were carefully prepared using boiled tap water. A few drops of culture containing dense *Paramecia* were transferred with special care to avoid making water currents as much as possible. The results given in Table II. are to be considered as the best parts of many series of experiments that were quite extensively carried out.

As the table shows, the majority of the animals always swam downward, *i. e.*, were positively geotropic, whenever transferred into any solution as well as in the control, *i. e.*, transfer into culture water. Among the substances used, perhaps, $CaCl_2$ solution "affected" the animals most. In this respect, Moore's observation seems to be confirmed, but it needs further consideration. In any solution, the animals that swam to the bottom did

not stay there very long. They resumed their negative geotropism within one hour. Sosnowski already observed the same and stated that "wenn sich darauf die Tiere wieder an der Oberfläche sammeln, kann man durch Zusatz einer neunen Quantitat Säure resp Alkali wieder die Ansammlung am Boden versanlassen." This statement is true. But it should be remembered that the same is also true when some tap water is added. It seems to the

TABLE II.

EFFECTS OF CHEMICALS.

	Room Temp. 20° C. Time.					
	Immediately after Treatment.	5 Minutes after Treatment.	10 Minutes after Treatment.	20 Minutes after Treatment.	30 Minutes after Treatment.	60 Minutes after Treatment.
Control.....	90% or more swim downward.	About 85% at bottom.	Number at bottom decreasing.	Scattered all over.	About 70% at upper part.	About 80% at top.
0.01 m. or 0.0133 m. $CaCl_2$.....	Abnormally active and 95% or more swim downward.	About 95% at bottom.	About 80% at bottom.	About 60% at lower part.	60% or more at upper part.	About 85% at top.
0.01 m. or 0.0133 m. Na_2CO_3 or $NaHCO_3$.....	About 95 % swim downward.	85% or more at bottom.	Scattered all over.	About 70% near top.	80% or more at top.	About same.
.05% HCl[1].....	90% or more swim downward.	70% or more at bottom.	Scattered all over.	About 60% at upper part.	About 70% near top.	About same.

writer that this means that the so-called reversible effect of chemicals on the negative geotropism of *Paramecium* is not specific but general. Moreover, the animals, whenever transferred to another receptacle without changing the solution, swim downward, and reversed animals do not stay long at the bottom.

There is, therefore, reason to believe that this is the same phenomenon that we met in the temperature experiments. The writer concludes, therefore, that mechanical "shock" or agitation of transferring, or chemical "shock," is chiefly responsible for so-called reversible effect of chemicals on the negative geotropism of *Paramecium* but not chemicals themselves.

Besides the chemicals mentioned above, sodium chloride,

[1] This was adopted as approximately Sosnowski's strength.

magnesium chloride, cane sugar, ethyl alcohol and chloroform in appropriate strength were tried but no better results were obtained.

IV. Summary and Conclusions.

1. The specific gravity of living *Paramecium caudatum* is between 1.0382 and 1.0393.

2. *Paramecium caudatum* contains protoplasmic materials of different gravities.

3. *Paramecium caudatum*, when strongly centrifuged, assumes a position with its anterior end directed away from the axis of the centrifuge. In so doing, it must be passively thrown by the centrifugal force, because the centrifugal force used was altogether too strong to be resisted by the animal. For this reason, its anterior end must be heavier than its posterior end. The negatively geotropic orientation, therefore, is an active process and the mechanical theory is not correct.

4. The whole organism seems to be a "statocyst," since the recovery of negative geotropism after centrifugalizing is synchronous with the reëstablishment of normal relations of substances in the cell.

5. So-called reversibility of the negative geotropism of *Paramecium caudatum* by temperature and by chemicals is extremely doubtful. Mechanical "shock" or agitation is chiefly responsible for the reversal one sees in such cases.

In conclusion the writer wishes to acknowledge his indebtedness to Professor E. P. Lyon, under whose direction this work was completed, and who gave valuable suggestions and criticism of the work and manuscript. His thanks are due also to Dr. J. F. McClendon, who gave his generous help and suggestions during the experiments.

NOTE ON THE GEOTROPISM OF PARAMECIUM.

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Harper's[1] criticism of my experiment demonstrating, as it seemed to me, that the anterior end of paramecium is the heavier and consequently that the negative geotropism of this animal is an active process was founded upon the assumption that when one begins to centrifuge the animals their geotropism is reversed and becomes positive. Thus the animals start, according to Harper, to move in the direction of the accelerating force and consequently away from the axis of the centrifuge. Harper's experiment by no means proves his contention and it always seemed to me, as Kanda brings out in the foregoing paper, that the quickness with which a high centrifugal force is developed in the centrifuge would prevent the animals from orienting themselves before they were thrown into the capillary tube where turning is impossible. However, this suggestion might not seem proof to some people; and I have, therefore, performed the following experiment:

A culture of paramecium was cooled almost to 0° C. At this temperature they move very sluggishly and show no distinct orientation to gravity. They gather at the bottom of a test tube due, I am sure to the fact that their specific gravity is greater than water and that they make no movement sufficient to keep themselves suspended. The centrifuge was prepared with capillaries such as Kanda and I have used but each one immersed in a larger tube containing ice. A drop of the cold paramecium culture was quickly transferred to the cold capillary tube and centrifuged. The animals were always precipitated with their anterior ends away from the axis. I am quite certain that the animals at this temperature make no reaction to gravity or agitation and that the proof may be considered definite that the head end of the animal is the heavier and that the ordinary geotropic orientation is an active process.

[1] For the literature see the preceding article by Kanda.

THE MICROVIVISECTION METHOD.

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CORNELL UNIVERSITY MEDICAL COLLEGE, NEW YORK CITY.

The dissection of living cells has hitherto proved inadequate for a study of the physical properties of protoplasm and of its structural components. This is owing largely to a lack of means for the manipulation of dissecting instruments under a sufficiently high magnification of the microscope.

Before and since Barber first devised his mechanical pipette-holder for the isolation of bacteria, various ingenious methods for the dissection of microscopic objects have been described. They all, however, fall short of Barber's method, because, with them, only comparatively low powers can be used, since the dissecting needles must operate *between* the objective and the tissue to be dissected. On the other hand, by using needles instead of micro-pipettes, Barber's instrument has been converted into an excellent microdissection apparatus. The needles operate in a shallow hanging drop containing the cells to be dissected which are pressed against the *undersurface of a coverslip* in a moist chamber. There being no obstacle above the coverslip, oil immersion lenses may be used for observation.

In an article appearing as long ago as 1859, Dr. H. D. Schmidt, of Philadelphia, describes in detail an excellent "microscopic dissector" consisting of a base to be fastened on the stage of a microscope with a number of clamps to hold instruments, each clamp possessing three movements controlled by screws. A lever fastened in one of the clamps holds the tissue in place. Fine scissors, knives or steel needles are fastened in the other clamps. By turning the various screws, the instruments can be brought into place and be operated with remarkable accuracy. Dr. Schmidt worked with the tissue, the instruments and the lower lens of the objective immersed in water or diluted alcohol. The full results of his investigations with this instrument were not published until 1869, owing to interruptions due to the Civil

War, during which his drawings and manuscript were burned in a conflagration in Washington. In his later paper he has two illustrations showing a liver cell being torn in two by needles.

In 1887 Chabry described an apparatus in which a glass needle was held in a sheath in such a way as to allow the needle to be pushed to any prescribed distance into the bore of a glass capillary tube. The bore of the capillary was made just large enough to admit a single ovum and hold it in place. By proper adjustments, the needle could be pushed to any depth in the ovum. The apparatus was placed on the stage of a microscope and Chabry was able to injure locally a *Strongylocentrotus* ovum while under observation. Chabry's instrument has been used for experimental embryological work and is described in detail in Ehrlich's "Enzyklopädie für microskopischen Technik," 1910.

In 1907 J. F. McClendon first described his "mechanical finger," which consisted of an ordinary Spencer mechanical stage with an additional screw to allow of movements in three different directions and a clamp to hold a needle or pipette. With this apparatus, McClendon was able to suck the nucleus out of a *Chætopterus* egg. In 1909 he described an improved form with which he dissected certain Protozoa.

In 1912 an apparatus was described by Tschachotin. It consists of a clamp attached to the side of the objective of a microscope. In the clamp is fastened a needle which curves so as to project under the objective and is so adjusted that the tip lies in the focus of the objective. The needle point is lowered into the object to be dissected simply by bringing the object into view under the microscope and lateral tears are made on moving the object with the slide by means of the mechanical stage.

In 1904 Barber first described his method of using a hanging-drop in a moist chamber for isolating microörganisms. A fuller account appeared in 1907 in which he first describes a mechanism for holding micropipettes and where he mentions Montrose T. Burrows as having assisted him much in its design. Elaborations of his instrument appeared in his papers of 1908 and 1911. In 1911 he suggested the possibility of the use of his instrument for cell dissection and for investigation on fertilization and heredity problems. His paper of 1914 is a detailed and precise descrip-

tion of his technique and is of the utmost value for the prospective worker in micro-dissection. As he developed his method principally for the use of pipettes and also because his latest paper appeared in a publication not readily obtainable, I have endeavored to give here a description of the method with modifications and new developments in the application of the method to microvivisection.[1]

A simple form of Barber's apparatus is shown in Fig. 1. The moist chamber, which is open at one end and with sides from 8 to

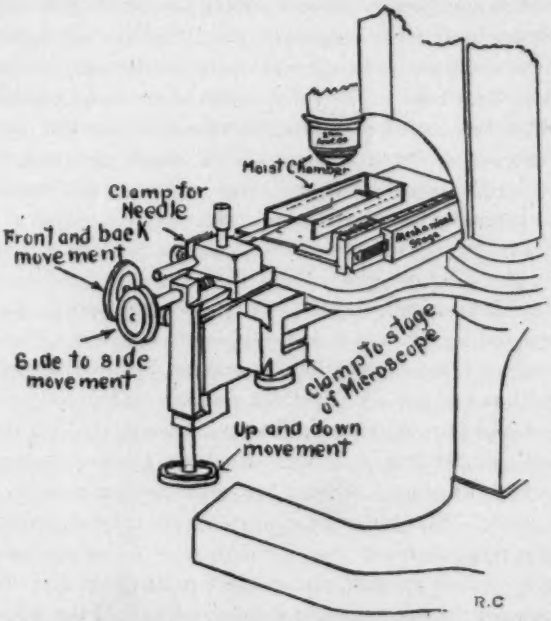

FIG. 1. Barber's single three-movement pipette holder, glass needle and moist chamber arranged to illustrate method of dissecting cells in a hanging drop under the highest magnification of the microscope. (Substage of miscroscope omitted in drawing.)

[1] In 1912, G. L. Kite first applied Barber's apparatus to microdissection, which quickly resulted in the publication of several papers. Kite was to have prepared an article on the method when his health unfortunately broke down. Publications on microdissection have appeared as follows: 1912, Kite (on the fertilization membrane) in *Science*, N. S., XXXVI.; Kite and Chambers (on chromosomes and nucleus) in *Science*, N. S., XXXVI. 1913, Kite (on permeability of cells) in BIOL.

12 mm. high, is placed on the microscope so that it may be moved about with the mechanical stage. The chamber is roofed over with a carefully cleaned coverslip, on the under surface of which, the specimen is mounted in a shallow hanging drop of a physiologically indifferent fluid. The dissecting needle is made by drawing out one end of a piece of glass tubing and bending it at right angles two or three millimeters from the pointed tip. The needle-holder, a mechanism allowing of three movements, is clamped to one side of the microscope stage and the needle is adjusted so that it may project into the moist chamber with its tip pointing up into the hanging drop. By proper adjustment the cell to be dissected and the point of the needle are brought into the same focal field. The three movements of the needle, permitted by the needle-holder, and the two movements of the moist chamber, by means of the mechanical stage, give the experimenter ample opportunity to carry on dissection under the highest magnification of the microscope.

The Instrument.

The holder accommodated to carry only one pipette or needle is illustrated in Fig. 1. It is useful in many ways and is comparatively inexpensive. Its disadvantage lies in the fact that the resistance of the cell-substance to the needle may overcome the inertia of the cell, so that, instead of tearing through the cell, the needle simply drags it about. However, by careful manipulation and by the possible use of a viscous dissection-medium much can be done. Many marine ova and germ cells in general are very soft in consistency and, if the drop in which they are immersed be shallow enough, the surface tension of the drop flattens them against the coverslip and is sufficient to hold the cell. The single holder can also be successfully used for injecting material into or extracting material out of a cell. In addition to the movement of the needle, manipulation is greatly aided by the

Bull., XXV., and (on protoplasm) in *Amer. Jour. Physiol.*, XXXII., 1914, Chambers (on the nucleus) in *Science*, XL.; Kite (structural transformations of blood cells) in *Jour. Inf. Dis.*, XV. 1915, Chambers (on the germ cell) in *Science*, XLI.; and (on protoplasm) in *Lancet-Clinic*, March 27, Cincinnati; Kite (on cell permeability) in *Amer. Jour. Physiol.*, XXXVII. 1917, Chambers (on protoplasm) in *Amer. Jour. Physiol.*, XLIII.; and (on the cell aster) in *Jour. Exp. Zoöl.*, XXIII.

use of the mechanical stage by means of which the moist chamber may be moved in two directions.

The double holder, formerly manufactured by the Fowler shops of the University of Texas, is twice as expensive as the single holder. It possesses, however, the advantage that two needles or pipettes may be used simultaneously. Each needle can be moved independently of the other and either needle may be replaced by a pipette. The manufacture of the holder has recently been duplicated by the mechanician of Wesleyan University under the direction of Dr. H. B. Goodrich with modifications of his and my suggestion. In setting up the apparatus, Dr. Goodrich introduced the useful innovation of having the shelf to which the instrument is clamped on a stand independent of the microscope. The two stands are then clamped together on a common base. The operator can thus shift the position of the instrument placing it at will in front of or on either side of the microscope.

Fig. 2 illustrates the double holder as I have it set up. The attachment of the instrument to the front of the microscope stage facilitates manipulation of the screws with both hands. In the illustration, a Spencer Lens Company lamp replaces the substage mirror. As the heat of the lamp, however, produces undue evaporation and subsequent condensation in the moist chamber, it is preferable to use the mirror. This necessitates raising the microscope-stand on a block of wood so that the mirror can be lowered below the microscope base so as to receive light unobstructed by the lower screws of the dissecting instrument.

The needle-carrier with its groove (Fig. 2, *a*), in which the arm of the needle lies, is twice as long as in Barber's original instrument. This extra length assists one in giving a straight even movement to the needle when it is being pushed into the moist chamber. After the needle has been thus adjusted by hand approximate to its proper position, the plate *b* is pushed over the arm of the needle and the screw *c* tightened to clamp the needle. Further adjustment of the needle is carried out by the screws *e*, *f*, *g* and *h* under the low and high powers of the microscope.

In preparing the shelf on which the instrument is to be clamped (Fig. 2, *j*) care must be taken that it be low enough to bring the top of the carrier flush with the upper surface of the microscope

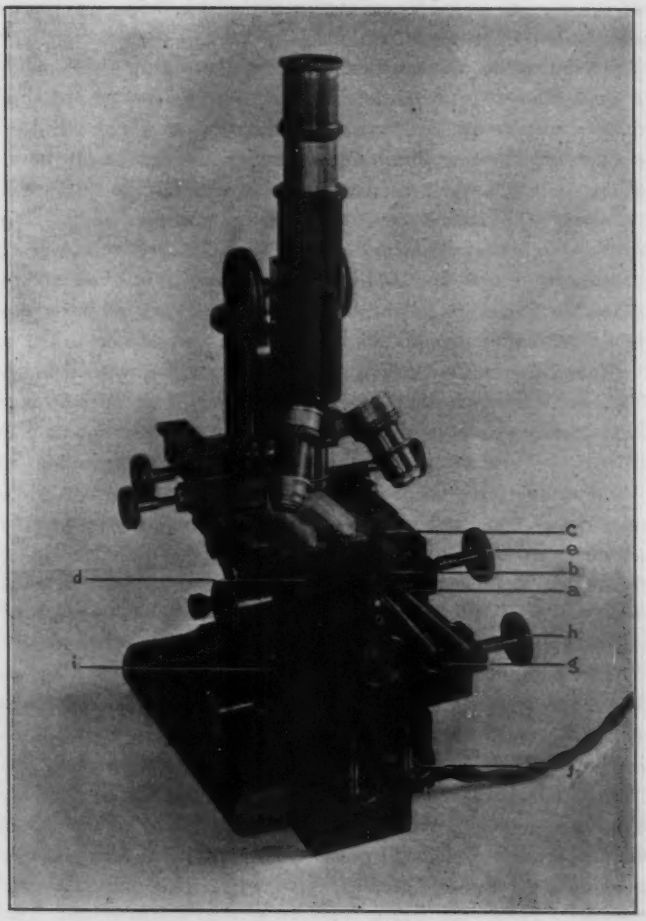

FIG. 2. Double pipette holder set up for microdissection. *a*, Carrier with groove to hold needle. *b*, brass plate which slips sideways over the groove in which needle lies. (The undersurface of the plate is covered with blotting paper to give it resiliency where it comes into contact with the glass needle.) *c*, set screw to clamp plate on carrier. *d*, screw which, on loosening, allows carrier to be set at any angle. *e–h*, screws to move the needle (*e*—side to side, *f*—up and down, *g*—in and out, *h*—side to side for both carriers together). *j*, shelf to which instrument is clamped.

stage. This is in order to enable one to use a moist chamber of the minimum height (see below).

An important factor in the manufacture of the microdissection instrument is that the two horizontal movements run true and keep the needle point always in the same plane. Otherwise, at a critical moment in the dissection, a reversal of a horizontal screw may either suddenly lower the needle-point out of focus or jam it against the coverslip and break it. Unfortunately, the last model put out by the University of Kansas is deficient in this respect. The existence of lost motion in the screws can be taken care of in the manipulation but if the needle tip does not run true in a horizontal plane the peace of mind of the operator may be sorely tried!

The Moist Chamber.

The moist chamber (Fig. 3) is a glass slide to which are cemented strips of glass in such a way as to form a box open at the top and at one end. For cement Canada balsam may be used.

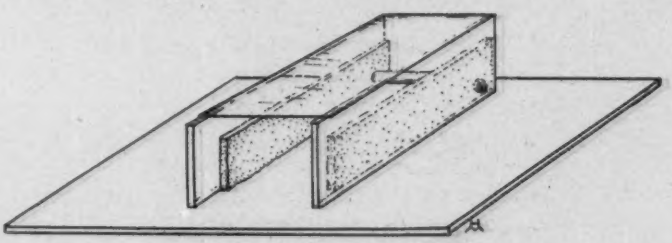

Fig. 3. Moist chamber for microdissection.

A shallow trough, to hold water, is made with a narrow glass strip set across the floor of the chamber 8 or 9 mm. from the closed end. This and other small strips set outside the chamber serve to reinforce the walls. Care must be taken that the upper edges of the chamber be even and level when placed on the stage of the microscope. A carefully grease-free cleaned coverslip with a hanging drop containing the objects to be dissected forms the roof of the chamber. The roof is sealed on with vaseline smeared along the upper edges of the walls of the chamber.

The height of the chamber is conditioned by the focal distance of the condensor used and by the minimum working room that

one must have in which to manipulate the needles. For routine work it may be well to have a chamber of fairly large dimensions disregarding the condensor as it is surprising how much illumination a condensor will give beyond its focal distance. However, for critical work, it is imperative to have the height of the chamber such that the objects on the undersurface of the coverslip be close to the focus of the condensor. My condensor has a working focal distance of almost 9 mm. and the chambers I use are 45 mm. long, 22 mm. wide and 9 mm. high.

The moist chamber is lined on the sides with blotting paper leading from the trough to carry the water along the length of the chamber so as to furnish a large moist surface. The liquid in the trough and the blotting paper should be isosmotic with the liquid used for the hanging drop so that evaporation be equalized and the hanging drop not become diluted.

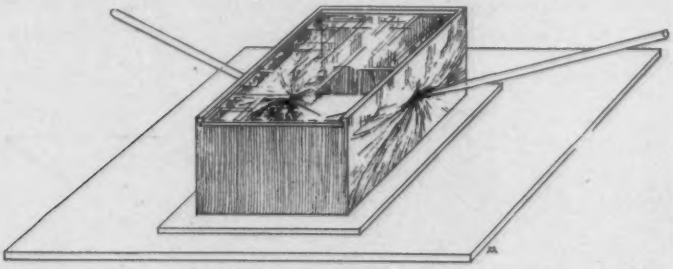

FIG. 4. Moist chamber for preliminary teasing of tissue.

For the dissection of the cellular elements of somatic tissues, it is necessary to do some preliminary teasing under an ordinary dissecting microscope. This should be done in a moist chamber of some kind. The tissue is teased on a coverslip which is then inverted and placed on the microdissection moist chamber.

A handy moist chamber (Fig. 4) can be made out of a shallow box with dimensions approximately 7 × 5 × 3 cm., with a top and bottom of glass and the two longer sides of thin rubber sheeting. The coverslip with the tissue to be teased is placed within the box and the teasing is carried out with needles projecting through the rubber sheeting. For the tissue of warm-blooded animals both the preliminary teasing and the microdissection should, of course, be carried on in warm boxes.

Tissue cultures are grown on square coverslips. The slips are then placed on the moist chamber for microdissection, the parts of the chamber not roofed over being previously covered with slips of glass.

The Needles.

These are made from glass tubing with an outside diameter of about 4–6 mm. and a lumen of 3–5 mm. The technic is readily mastered with soft glass tubing but needles made of hard glass are more durable.

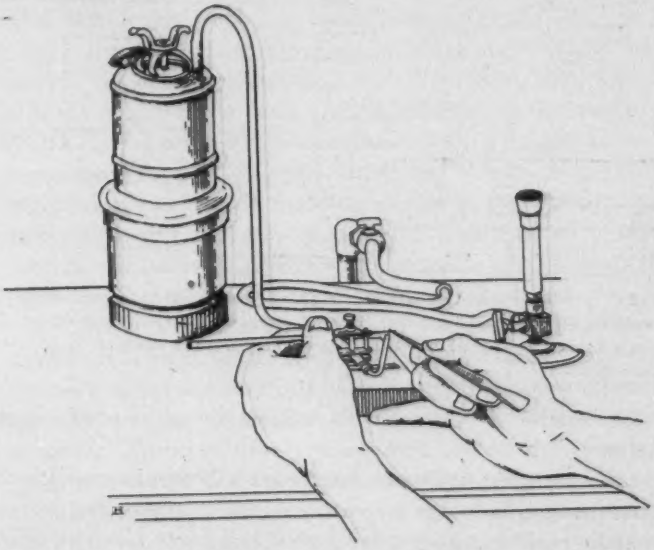

Fig. 5. Method of making the capillary pipettes and microdissection needles.

The needle points are made over a minute flame. A serviceable micro-burner with an acetylene generator is shown in Fig. 5. The microburner is made out of a piece of hard glass tubing bent almost at right angles and with one end closed except for the smallest possible aperture that will retain a flame. This is done by heating the end and pinching it with a pair of forceps. When set up the tip of the burner should be at a height of about 5–6 cm. above the surface of the table. The size of the flame is regulated by the screw pinch-cock set on the rubber tube connecting the burner with the gas generator.

As a source for the flame acetylene is to be preferred to ordinary illuminating gas because with the former a narrower flame can be obtained without undue clogging. The acetylene generator in the figure is part of an old bicycle lamp. A more convenient way is to have the acetylene in a small compression tank which can be recharged at a very small cost. If, however, ordinary gas is to be used, one may much improve the gas by passing it through alcohol or benzene.

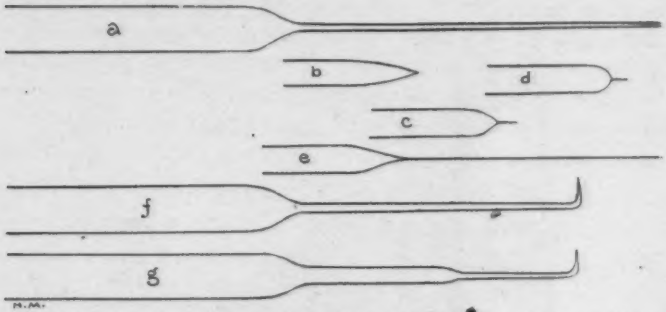

FIG. 6. Stages in making needles. *a*, glass tube with one end drawn out into a capillary. *b*, good tapering point. *c* and *d*, serviceable points. *e*, tip drawn out into a hair. *f*, completed microdissection needle. *g*, needle made on a relatively stout shank.

The method of procedure in making the glass needle is as follows:

1. In an ordinary Bunsen burner (or a Mecca burner if hard glass be used) draw out one end of a piece of glass tubing into a straight capillary about 0.3–0.5 mm. in outside diameter (Fig. 6, *a*). Prepare a supply of such pieces with the capillary ends at least 6 cm. long.

2. Lower the flame of the microburner to the smallest flame that will remain lighted. The microburner should be protected from draughts of air and in semi-darkness so that the flame may show up to best advantage.

3. Hold the shank of the glass tube in the left hand and, with a pair of fine forceps held in the right, grasp the capillary at a point about 5 cm. from the shank. Bring the portion of the capillary next the forceps over the flame and at right angles to it at a point just over the flame (Fig. 5). Pull gently with the

forceps and, when the glass begins to soften, lift it slowly from the flame and pull with the forceps slightly more than at first, but not too strongly. The hands should remain on the table during the process and the pulling and lifting done by turning them slightly outward. The capillary will separate with a slight tug—a feeling much like that experienced when a taut thread, held in the fingers, is parted in a small flame. If the point is properly made, it will appear as shown in Fig. 6, *b*, *c* and *d*. This capillary has sufficient rigidity and it comes to a very fine point. The tip is closed, but the lumen extends almost to the end. It is evident that everything depends upon the amount of heat used and the timing of the pull, and that these must vary slightly with the height of the flame and the diameter of the capillary. With a little experience, one can usually tell when a proper point is made by the peculiar feeling described above. If too little heat is used and the pull made too suddenly, the capillary may part with a snap and the tip be found broken off short often forming a serviceable pipette. If too much heat is used, the capillary is apt to be drawn into a long hair (Fig. 6, *e*).

4. After a suitable point is made, the end of the capillary is turned up at right angles (Fig. 6, *f*). This is done by holding the point of the capillary just back of the point above the small flame and pushing up the end with the tip of the forceps or with a needle. Not more than 5 mm. should be turned if it is to be used in a chamber not over 8 mm. in height. If a good point is made, *e. g.*, Fig. 6, *b*, it is not necessary to turn the end at exactly right angles. A pipette is made by jamming the tip of a needle against the cover glass in the moist chamber to break off the point.

The forceps used in the making of the needles should be of a size that can be firmly grasped in the hand. The fine tips should be accurately set and almost parallel one another for a few millimeters. In order to give the tips a good, grasping, resilient surface I dip them, before using, in a small vial of Sandarac varnish which may be obtained from a dentists' supply dealer. As the varnish burns if brought too close to the flame, it is well to use a second forceps or a needle for bending the glass capillary.

Fine points are not readily made from capillaries of a diameter

greater than .05 mm. Therefore, if the dissection requires a comparatively stiff shank for the dissecting needle, start with a glass tube possessing a thick capillary and draw out the tip into a thinner capillary. This thinner capillary may then be used for making the needle point (Fig. 6, *g*).

The facility in making the needle-points over the microburner increases, of course, with practice. In my case, I make at times twenty or thirty within an hour, at other times none, or only one or two at the most. A supply may be kept on hand on a stand made of two horizontal layers of wire netting, the meshes of which admit the base of the needles. It is well to mark off the stand into compartments to receive the various types of needles that are made.

Another good method for making the needle points is that of Chabry ('87). The tip of a capillary pipette is brought into contact with a heated mass of glass (or any incandescent body to which glass will adhere) and suddenly drawn away. For an incandescent body Chabry used the blade of a platinum knife of a surgical instrument for thermocautery. The glass capillary was held by the operator in a groove on a stand a few centimeters from the platinum blade, which was fastened so as to be immovable. An assistant controlled the heating of the platinum blade. As soon as it heated to a dull glow, the operator slid the capillary on its groove until the tip touched the glowing metal when it was instantly slid back. The sliding of the capillary in a stationary groove insures a tapering to a point in a straight line with the long axis of the capillary. In his paper Chabry discusses this method in some detail, pointing out ways of avoiding difficulties in the technique ('87, pp. 175–178). Chabry also suggested the use of insect mouth parts, annelidan bristles and the spicules of sponges for the tips of microdissection needles. Barber suggested fine-pointed needle crystals, or the sharp, stiff hairs taken from the body of a house fly. A pipette with a fairly large opening is made and the hair or crystal is drawn partially into it. The fine point projecting from the tip of the pipette is then used as a probe or dissecting needle. Such needles could be used only on very delicate soft objects.

Needle points may also be made by grinding one end of a fine

wire. To produce chemical effects in a cell, McClendon ('09) used a copper wire ground to a point and further sharpened by erosion in acid. The chemical injury produced by metal needles limit their use for the dissection of living cells.

For experiments in cutting cells, one must take into account the fact that many cells will lose their more or less fluid contents if a gap be torn in their surface. If, however, the side of a fine needle can be brought to press the cell against the coverslip, a constriction will result which may cut the cell into two intact pieces. For cutting soft-bodied ova and protozoa this can be done with a form of needle depicted in Fig. 7. The needle tip

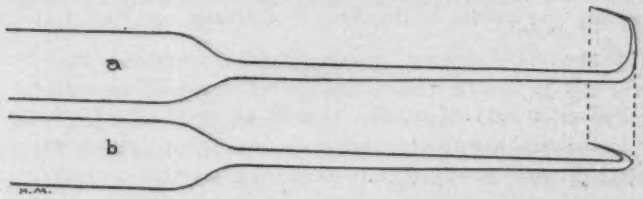

FIG. 7. Needle with tip curved back to almost an horizontal plane. *a*, lateral view. *b*, view from above.

is bent back rather than forward to lessen the chances of breaking the tip when adjusting the needle in the moist chamber and also when the needle needs to be cleaned (see below). The tip should not point directly back but slant off to one side (see Fig. 7, *b*), so that the shank of the capillary will not be in the way of the light coming up from below. The angle at which the needle is bent must be ascertained by successive trials, for, if it be bent too near to a horizontal plane, the tapering tip will bend away from the cell instead of cutting into it. If the angle be too far from the horizontal plane, the cell will slip along the needle and escape. When the proper angle is obtained, one can cut *Arbacia* and *Asterias* ova into pieces of almost any required size with considerable ease. A clean cut through an unfertilized sea-urchin egg or a fertilized egg, before the fertilization membrane has become too tough, can be best made by bringing the egg between the horizontal arm of the needle and the lower surface of the hanging drop (Fig. 8). On lowering the needle out of the drop,

the arm of the needle passes through the egg cutting it cleanly in two.

A good needle, when once made and proven to be resilient and strong, may be used repeatedly. Mucinous material which soon

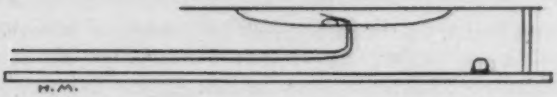

FIG. 8. Side view of moist chamber to show method of cutting an egg cell in two with needle shown in Fig. 7.

covers its tip so as to render it useless can be got rid of by immersing the needle successively in a strong acid and a strong alkali.

CELL INJECTION.

Barber, in his two papers ('11 and '14) gives a full description of his admirable mercury method for injection. With it one can inject microscopically minute doses of a solution into any part of a cell. The advantage of the pressure produced by the mercury over that by air lies in the slow driving force of expanding mercury which can be almost instantly arrested when the dose injected is considered sufficient. On the other hand, if air pressure be used, the air in the injection tube will compress enormously as the resistance of the bore at the tip of the pipette is too great to overcome. If the injection does occur it may go with a rush usually followed by a volume of air which destroys the cell being experimented upon. Notwithstanding this, it is surprising what delicate work may be done simply with air pressure. An example of this is the extraction of the nucleus from a mature *Arbacia* egg and its subsequent injection into another egg. For the injection of substances, however, where the amount must be strictly controlled, the mercury method is essential.

The extraction of material out of a cell is comparatively easy to carry out, as the capillary attraction in the lumen of a micropipette (the inside diameter of which may be anywhere from 2 micra up) is quite sufficient for the purpose. For injection purposes the difficulty lies in securing the force to overcome this capillary attraction.

Source of Light and Lenses.

A most serviceable light for microscopic work is the 100-watt horseshoe filament, Edison, Mazda, nitrogen lamp with the blue daylight glass. This lamp, when set in a frosted milk-white glass globe, gives a white light very restful to the eye.

In selecting lenses care should be taken to procure those possessing the maximum focal length together with the greatest numerical aperture. For this reason the Zeiss apochromatic 4 mm. dry lens with a N. A. 0.95 and the 3 mm. oil immersion lens with N. A. 1.40 are excellent for microdissection or for the study of living tissue in general. It is to be remembered that the equivalent focus of a lens does not correspond with the working focal distance.

A good substage condensor with a working focal distance of 8–9 mm. can be made by removing the top lens of the Leitz triple-lens, centering condensor. The beam of light issuing from it completely fills the back lens of a 4 mm. objective with N. A. 0.65 and more than half of a 1/12 oil immersion objective with a N. A. 1.30. This gives enough definition for such detailed cytological work as the study of the chromomeres in grasshopper germ-cells and of the types of protoplasmic granules in marine ova. One may obtain very good condensors with a long working focal distance from those used in projection apparatus where a cooling trough is inserted between the condensor and the microscope stage.

Setting up the Instrument.

The instrument is first clamped to the microscope and the moist chamber, with the edges of its walls smeared with vaseline, placed in the mechanical stage. The needle is then adjusted as follows: Place the arm of the needle in the groove of the carrier with the dissecting tip extending into the moist chamber. Push the plate of the carrier (*b*, in Fig. 2, page 126) over the arm of the needle and tighten the set screw *c* just enough to allow one to push the needle with a straight and even movement. The needle is now adjusted by hand so as to bring the tip within the field of a low power objective. The set screw is then tightened to clamp the needle in place. Further centering of the needle is made by

the screws *e*, *f*, *g* and *h*, under the low and high powers of the microscope. Finding the tip of the needle under high powers is facilitated by using an ocular containing cross hairs.

The coverslip with its drop containing the tissue to be dissected is now inverted over the moist chamber. The cells to be dissected are brought into the field of the microscope. The tip of the needle is then raised by the screw *c* until brought into view first under low and then under high power. The apparatus is now ready for dissection.

In conclusion I wish to suggest to the prospective worker in this field that he use the utmost conservatism in his interpretations of observed effects produced in microdissection. One must constantly keep in mind the extraordinary instability of living cell structures.

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